stemming from physical and physiological damage the cells suffer in the process of cryopreservation. Finally, there is an ongoing need for a reducing cellular plasticity, necroptosis, or necrosis that results from physical and physiological damage cells incur in the course of cell manipulations *in vitro*, during liberation from tissue.

[8] The composition and methods thereof disclosed herein addresses these needs, wherein use of the composition may reduce the incidence of necroptosis or necrosis during cryopreservation, and may treat, prevent, inhibit, or reduce the incidence of cellular plasticity in a plurality of cells.

SUMMARY

[8a] In one aspect of the present disclosure, there is provided a cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound.

[8b] In another aspect of the present disclosure, there is provided a method of cryopreservation, the method comprising the steps of:

(a) bringing a plurality of cells in contact with a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound; and

(b) cooling the composition comprising the plurality of cells of step (a),

wherein the necroptosis inhibitor compound comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

[8c] In a further aspect of the present disclosure, there is provided a use of a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound, wherein the necroptosis inhibitor compound comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, or necroptosis or necrosis

in a plurality of cells.

[8d] In another aspect of the present disclosure, there is provided a composition for use in preventing excessive necrosis and necroptotic cell death, the composition comprising at least one inhibitor of necrosis- or necroptosis-inducing damage-associated molecular patterns (DAMPs).

[9] In one example, disclosed herein is a composition comprising a necroptosis inhibitor and a Bax channel inhibitor. In another example, the necroptosis inhibitor comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and the Bax channel inhibitor comprises a 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

[10] In a related example, a necroptosis inhibitor comprises a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one compound, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin compound, 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2-hydroxyethanone compound, (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide compound, methyl-thiohydantoin-tryptophan compound, (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide compound, 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl)phenyl)urea compound, or 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt of said necroptosis inhibitor compound.

[11] In another related example, the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.2nM – 2μM. In another related example, the concentration of said 3,6-Dibromo-α-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound, or

analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.5nM-50μM.

[12] In another example, a composition disclosed herein further comprises a nicotinamide adenine dinucleotide (NAD), an adenosine triphosphate (ATP), a cyclosporin A, or a superoxide dismutase, or any combination thereof. In a related example, the superoxide dismutase comprises a manganese superoxide dismutase or a zinc superoxide dismutase. In another related example, the concentration of said NAD is about 5nM – 500μM. In another related example, the concentration of said ATP is about 10nM – 1mM. In another related example, the concentration of said cyclosporin A is about 1nM – 1mM. In another related example, the concentration of said superoxide dismutase is about 0.001– 100 Kunitz Units (KU).

[13] In another example, a composition disclosed herein comprises about 20 nM 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione), about 5nM 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride, about 0.05 mM nicotinamide adenine dinucleotide (NAD), about 0.01 μM adenosine triphosphate (ATP), about 0.01μM cyclosporin A, and about 0.1 Kunitz Unit manganese superoxide dismutase or zinc superoxide dismutase.

[14] In a related example, a composition described herein further comprises a cryoprotective agent. In another related example, the cryoprotective agent comprises DMSO, or serum, or any combination thereof.

[15] In a related example, a composition described herein further comprises a pharmaceutically acceptable excipients or carriers.

[16] In one example, disclosed herein is a method of cryopreservation, the method comprising the steps of: bringing a plurality of cells in contact with a composition comprising a necroptosis inhibitor and a Bax channel inhibitor; and cooling the composition comprising the plurality of cells of step (a), wherein the necroptosis inhibitor comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

[17] In one example, disclosed herein is a method of treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, or necroptosis or necrosis in a plurality of cells, said method comprising the step of: bringing said plurality of cells in contact with a composition comprising a

necroptosis inhibitor and a Bax channel inhibitor, wherein the necroptosis inhibitor comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

[18] In a related example, the necroptosis or necrosis is associated with aging or disease. In another related example, the disease is myocardial infarction, diabetes secondary to beta-cell necroptosis, cholestatic liver disease, stroke, organ ischemia, ischemia-reperfusion injury, liver disease, necrosis from cancer chemotherapy or radiation therapy, traumatic brain injury, necrotizing pancreatitis, pathogen-induced necroptosis, inflammation, or neurodegenerative disease.

[19] In a related example, the treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, or necroptosis or necrosis, comprises *in vitro* or *in vivo* treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, necroptosis, or necrosis. In another related example, the plurality of cells comprises tissue culture cells, primary cells, egg cells, a tissue, or an organ or a portion thereof, or any combination thereof. In another related example, the tissue culture cells or primary cells comprise stem cells, adult cells, transdifferentiated cells, dedifferentiated cells, or differentiated cells, or any combination thereof. In another related example, the plurality of cells comprises human cells or animal cells. In another related example, the bringing in contact is *in vivo*, *ex vivo*, or *in vitro*. In another related example, the bringing in contact *in vivo* or *ex vivo* comprises perfusion of an animal or a portion thereof, an organ or a portion thereof, or a tissue. In another related example, the perfusion is cardiac perfusion. In another related example, the bringing in contact *in vitro* comprises immersing said plurality of cells in said composition, supplementing a growth media of said plurality of cells with said composition, perfusing said plurality of cells with said composition via non-thermal reversible electroporation, or perfusing said plurality of cells with said composition in a bioreactor.

[20] In a related example, methods disclosed herein further comprise a step of physical, chemical, or thermodynamic manipulation of said plurality of cells. In a related example, the thermodynamic manipulation comprises heating or cooling said plurality of cells. In a related example, the cooling comprises cryopreservation or a freeze-thaw cycle, or a combination thereof.

[21] In a related example, methods disclosed herein prevent, inhibit, or reduce necrosis or

necroptic death of said plurality of cells during said cryopreservation or a freeze-thaw cycle thereof, compared with an uncontacted plurality of cells.

[22] In a related example, the methods disclosed herein protect said plurality of cells from physical damage during said cryopreservation or freeze-thaw cycles thereof. In another related example, the methods enhance growth potential of said plurality of cells during cryopreservation compared with uncontacted cells. In another related example, the methods prevent oxidative damage to said plurality of cells during cryopreservation or freeze-thaw cycles thereof. In another related example, the methods prevent ischemia of said cells during cryopreservation or freeze-thaw cycles thereof. In another related example, the methods inhibit necrosis pathway signaling. In another related example, the methods prevent, inhibit, or reduce changes in the differentiation state of said cells, thereby stabilizing the identity of said cells compared with uncontacted cells. In another related example, the methods induce a state of metabolic suspension in said cells. In another related example, the metabolic suspension comprises reversible cessation of oxygen metabolism. In another related example, the methods improve viability or latent viability of said cells compared with an uncontacted plurality of cells.

[23] In another example, in methods disclosed herein the necroptosis inhibitor compoundcomprises a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin, a methyl-thiohydantoin-tryptophan compound, a 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2- hydroxyethanone, a (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide, a (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide, a 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl)phenyl)urea, or a 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one, or a derivative, isomer, or pharmaceutically acceptable salt of any one of said necroptosis inhibitor compounds.

[24] In a related example, in the methods disclosed herein the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.2nM – 2μM, and wherein the concentration of the 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol

dihydrochloride compound, or analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.5nM – 50μM.

[25] In another related example, in the methods disclosed herein the composition further comprises, a nicotinamide adenine dinucleotide (NAD), an adenosine triphosphate (ATP), a cyclosporine A, a manganese-superoxide dismutase, or a zinc-superoxide dismutase, or any combination thereof. In another related example, in the methods disclosed herein, the concentration of said NAD is about 5nM – 500μM, the concentration of said ATP is about 10nM – 1mM, the concentration of said cyclosporin A is about 1nM – 1mM, and the concentration of said manganese-superoxide dismutase or said zinc-superoxide is about 0.001KU-100.0KU.

[26] In another related example, in the methods disclosed herein, the concentration of necroptosis inhibitor 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound, or the analog, a derivative, a isomer, or a pharmaceutically acceptable salt thereof is about 20nM, the concentration of Bax channel inhibitor 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride compound, or the analog, the derivative, the isomer, or the pharmaceutically acceptable salt thereof is about 5nM, the concentration of said NAD is about 0.05 nM, the concentration of said ATP is about 0.01μM, the concentration of said cyclosporin A is about 0.01μM, and the concentration of said manganese-superoxide dismutase or the zinc-superoxide is about 0.1KU.

[27] In a related example, in the methods disclosed herein, the composition further comprises a cryoprotective agent. In another related example, the cryoprotective agent comprises DMSO, or serum, or any combination thereof.

[28] In a related example, in the methods disclosed herein, the composition further comprises a pharmaceutically acceptable carriers or excipients.

BRIEF DESCRIPTION OF THE DRAWINGS

[29] The following drawings form part of the present specification and are included to further demonstrate certain embodiments of the present disclosure, the methods described herein may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[30] **Figures 1A-C.** Tissue dissection and cell manipulation produces reactive oxygen species (ROS) (detection of Nox1), apoptosis (detection of CASP3), necrosis (detection of staining with

CLAIMS

What is claimed is:

1. A cryopreservation composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound.

2. The cryopreservation composition of claim 1, wherein said necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and said Bax channel inhibitor comprises a 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

3. The cryopreservation composition of claim 1, wherein said necroptosis inhibitor compound comprises a 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione, a 3-p-Methoxyphenyl-5,6-tetramethylenothieno[2,3-d]pyrimidin-4-one-2-mercaptoethylcyanide compound, a (Z)-5-((3-(4-Fluorophenyl)-1H-pyrazol-4-yl)methylene)-2-imino-3-(thiazol-2-yl)thiazolidin-4-one compound, a 5-(Indol-3-ylmethyl)-(2-thio-3-methyl)hydantoin compound, 1-([3S,3aS]-3-[3-fluoro-4-[trifluoromethoxy]phenyl]-8-methoxy-3,3a,4,5-tetrahydro-2H-benzo[g]indazol-2-yl)-2- hydroxyethanone compound, (S)-N-(1-[2-chloro-6-fluorophenyl]ethyl)-5-cyano-1-methyl-1H-pyrrole-2-carboxamide compound, methyl-thiohydantoin-tryptophan compound, (E)-N-(4-(N-(3-Methoxypyrazin-2-yl)sulfamoyl)phenyl)-3-(5-nitrothiophene-2-yl)acrylamide compound, 1-(4-(4-Aminofuro[2,3-d]pyrimidin-5-yl)phenyl)-3-(2-fluoro-5-(trifluoromethyl) phenyl)urea compound, or 2,2-dimethyl-1-(5(S)-phenyl-4,5-dihydro-pyrazol-1-yl)-propan-1-one compound, or an analog, derivative, isomer, or pharmaceutically acceptable salt of said necroptosis inhibitor compound.

4. The cryopreservation composition of any one of claims 1-3, wherein the concentration of the 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or said analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.2nM – 2μM.

5. The cryopreservation composition of any one of claims 1-4, wherein the concentration of said 3,6-Dibromo-α-(1-piperazinylmethyl)-9H-carbazole-9-ethanol dihydrochloride compound, or analog, derivative, isomer, or pharmaceutically acceptable salt thereof is about 0.5nM-50μM.

6. The cryopreservation composition of any one of claims 1-5, further comprising a nicotinamide adenine dinucleotide (NAD), an adenosine triphosphate (ATP), a cyclosporin A, or a superoxide dismutase, or any combination thereof.

7. The cryopreservation composition of claim 6, wherein said superoxide dismutase comprises a manganese superoxide dismutase or a zinc superoxide dismutase.

8. The cryopreservation composition of any one of claims 6-7, wherein the concentration of said NAD is about 5nM – 500μM.

9. The cryopreservation composition of any one of claims 6-8, wherein the concentration of said ATP is about 10nM – 1mM.

10. The cryopreservation composition of any one of claims 6-9, wherein the concentration of said cyclosporin A is about 1nM – 1mM.

11. The cryopreservation composition of any one of claims 6-10, wherein the concentration of said superoxide dismutase is about 0.001– 100 Kunitz Units (KU).

12. The cryopreservation composition of any one of claims 6-11, comprising about 20 nM 5-((7-Chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione), about 5nM 3,6-Dibromo-a-(1-piperazinylmethyl)-9H- carbazole-9-ethanol dihydrochloride, about 0.05 mM nicotinamide adenine dinucleotide (NAD), about 0.01 μM adenosine triphosphate (ATP), about 0.01μM cyclosporin A, and about 0.1 Kunitz Unit manganese superoxide dismutase or zinc superoxide dismutase.

13. The cryopreservation composition of any one of claims 1-12, further comprising a cryoprotective agent.

14. The cryopreservation composition of claim 13, wherein said cryoprotective agent comprises DMSO, or serum, or any combination thereof.

15. The cryopreservation composition of any one of claims 1-14, further comprising pharmaceutically acceptable excipients or carriers.

16. A method of cryopreservation, the method comprising the steps of:

(a) bringing a plurality of cells in contact with a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound; and

(b) cooling the composition comprising the plurality of cells of step (a),

wherein the necroptosis inhibitor compound comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof.

17. Use of a composition comprising a necroptosis inhibitor compound and a Bax channel inhibitor compound, wherein the necroptosis inhibitor compound comprises a 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, and wherein the Bax channel inhibitor comprises 3,6-dibromo-α-(1-piperazinylmethyl)-9*H*-carbazole-9-ethanol dihydrochloride or an analog, derivative, isomer, or pharmaceutically acceptable salt thereof, in the manufacture of a medicament for treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, or necroptosis or necrosis in a plurality of cells.

18. The use of claim 17, wherein said necroptosis or necrosis is associated with aging or disease.

19. The use of claim 18, wherein said disease is myocardial infarction, diabetes secondary to beta-cell necroptosis, cholestatic liver disease, stroke, organ ischemia, ischemia-reperfusion injury, liver disease, necrosis from cancer chemotherapy or radiation therapy, traumatic brain injury, necrotizing pancreatitis, pathogen-induced necroptosis, inflammation, or neurodegenerative disease.

20. A composition for use in preventing excessive necrosis and necroptotic cell death, the composition comprising at least one inhibitor of necrosis- or necroptosis-inducing damage-associated molecular patterns (DAMPs).