CLAIMS

What is claimed is:

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

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

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

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

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

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

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

15. The 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. 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 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.

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

19. The method 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. The method of any one of claims 17-19, wherein said treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, or necroptosis or necrosis, comprises *in vitro* or *in vivo* or *ex vivo* treating, preventing, inhibiting, or reducing the incidence of cellular plasticity, necroptosis, or necrosis.

21. The method of any one of claims 16-20, wherein 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.

22. The method claim 21, wherein said tissue culture cells or primary cells comprise stem cells, adult cells, transdifferentiated cells, dedifferentiated cells, or differentiated cells, or any combination thereof.

23. The method of any one of claims 16-22, wherein the plurality of cells comprises human cells or animal cells.

24. The method of any one of claims 16-23, wherein bringing in contact is *in vivo*, *ex vivo*, or *in vitro*.

25. The method of claim 24, wherein 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.

26. The method of claim 25, wherein said perfusion is cardiac perfusion.

27. The method of claim 24, wherein 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.

28. The method of any one of claims 16-27, further comprising the step of physical, chemical, or thermodynamic manipulation of said plurality of cells.

29. The method of claim 28, wherein the thermodynamic manipulation comprises heating or cooling said plurality of cells.

30. The method of claims 29, wherein said cooling comprises cryopreservation or a freeze-thaw cycle, or a combination thereof.

31. The method of any one of claims 16 and 21-30, wherein said method prevents, inhibits, or reduces 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.

32. The method of any one of claims 16 and 21-31, wherein said method protects said plurality of cells from physical damage during said cryopreservation or freeze-thaw cycles thereof.

33. The method of any one of claims 16 and 21-32, wherein said method enhances growth

potential of said plurality of cells during cryopreservation compared with uncontacted cells.

34. The method of any one of claims 16 and 21-33, wherein said method prevents oxidative damage to said plurality of cells during cryopreservation or freeze-thaw cycles thereof.

35. The method of any one of claims 16 and 21-34, wherein said method prevents ischemia of said cells during cryopreservation or freeze-thaw cycles thereof.

36. The method of any one of claims 16-35, wherein said method inhibits necrosis pathway signaling.

37. The method of any one of claims 16-36, wherein said method prevents, inhibits, or reduces changes in the differentiation state of said cells, thereby stabilizing the identity of said cells compared with uncontacted cells.

38. The method of any one of claims 16-37, wherein said method induces a state of metabolic suspension in said cells.

39. The method of claim 38, wherein said metabolic suspension comprises reversible cessation of oxygen metabolism.

40. The method of any one of claims 16-39, wherein said method improves viability or latent viability of said cells compared with an uncontacted plurality of cells.

41. The method of any one of claims 16-40, wherein the necroptosis inhibitor comprises 5-((7-chloro-1H-indol-3-yl)methyl)-3-methyl-2,4-imidazolidinedione compound, 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 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 inhibitors.

42. The method of any one of claims 16-41, 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, 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.

43. The method of any one of the claims 16-42, wherein 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.

44. The method of claim 43, wherein the concentration of said NAD is about 5nM – 500μM, the concentration of said ATP is about 10nM – 1mM, the concentration of said cyclosporine A is about 1nM – 1mM, and the concentration of said manganese-superoxide dismutase or said zinc-superoxide is about 0.001KU-100.0KU.

45. The method of claim 44, wherein the concentration of necroptosis inhibitor compound 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 cyclosporine A is about 0.01μM, and the concentration of said manganese-superoxide dismutase or the zinc-superoxide is about 0.1KU.

46. The method of any one of claims 16-45, wherein the composition further comprises a cryoprotective agent.

47. The method of claim 46, wherein said cryoprotective agent comprises DMSO, or serum, or any combination thereof.

48. The method of any one of claims 16-47, wherein the composition further comprises a pharmaceutically acceptable carriers or excipients.

49. 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).

50. The composition of claim 49, wherein said inhibitors comprise antibodies, proteins, peptides, small molecules or any combination thereof.

51. The composition of any one of claims 49-50, wherein said DAMPs comprise, lipoxygenases inhibitors, sphingomyelinases inhibitors, phospholipases inhibitors, ceramidases inhibitors pattern-recognition receptors (PRRs) ligands, Toll-like receptors (TLRs) ligands, retinoic acid-inducible gene I-like receptors (RIG-I-like) receptors (RLRs) ligands, nucleotide-binding domain and leucine-rich repeat containing molecules (NLRs) ligands, C-type lectin receptors (CLRs) ligands, or death receptors ligands.

52. The composition of claim 51, wherein said ligands comprise high mobility group box 1 protein (HMGB1), heat-shock proteins (HSPs), ribonucleoproteins (RNPs), messenger ribonucleic acids (mRNAs), high mobility group nucleosome binding domain 1 (HMGN1), hyaluronan, biglycan, heparin sulfate, U1 small nucleolar ribonucleoprotein (snRNP), mitochondrial DNA, or double-stranded RNA (dsRNA).

53. The composition of claim 51, wherein said death receptors comprise tumor necrosis factor receptor 1 (TNFR1), tumor necrosis factor receptor 2 (TNFR2), CD95/FAS receptor, TNF-related apoptosis-inducing ligand receptor 1 (TRAILR1), and TRAILR2.

54. The composition of claim 51, wherein said death receptor ligands comprise, deubiquitylating enzymes, FAS-associated protein with a death domain (FADD), riboflavin kinase (RFK), tumor necrosis factor, TNFR2, FAS ligand (FASL or CD95L), and TNFR-associated death domain (TRADD).

55. The composition of claim 54 wherein said deubiquitylating enzymes comprise, A20 (TNFAIP3), OTUD7b, cylindromatosis (cylD) and USP21.

56. The composition according to any one of the claims 49-55, further comprising pharmaceutically acceptable excipients or carriers comprising pH stabilizers, sugars, amino acids, or antibiotics, or any combination thereof.