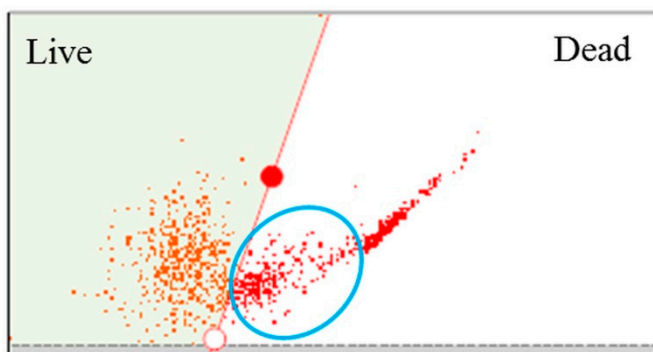


**Fig. S1.** Programmed and recorded freezing curve of bull semen; dotted line – programmed rate of freezing, continuous line – recorded rate of freezing.



**Fig. S2.** Representative picture of viability measurement of thawed bull semen, showing three populations of spermatozoa – live, dead, and third intermediate population, marked with blue oval.

**Table S1.** Mixing and dying scheme of samples of spermatozoa extract and supernatant of thawed bull sperm from young and adult bulls; N = 6 for each group

Gel #	Cy2	Cy3	Cy5
1	Pooled Standard	Young 1	Adult 3
2	Pooled Standard	Adult 1	Young 4
3	Pooled Standard	Young 2	Adult 4
4	Pooled Standard	Adult 2	Young 5
5	Pooled Standard	Young 3	Adult 6
6	Pooled Standard	Adult 5	Young 6

**Table S2.** Molecular and cellular functions performed by proteins changing in adult bulls cryopreserved spermatozoa in comparison to young bulls cryopreserved spermatozoa

Categories	Diseases or Functions Annotation	p-Value	Molecules	# Molecules
Energy Production, Nucleic Acid Metabolism, Small Molecule Biochemistry	Synthesis of ATP	6.99E-07	ALDOA, HSPD1, PKM, SOD1	4
Free Radical Scavenging, Molecular Transport	Quantity of hydrogen peroxide	1.39E-06	PARK7, PRDX5, SOD1	3
Free Radical Scavenging, Molecular Transport	Accumulation of hydrogen peroxide	1.22E-05	PRDX5, SOD1	2
Free Radical Scavenging	Modification of hydrogen peroxide	3.39E-05	PRDX5, SOD1	2
Free Radical Scavenging, Small Molecule Biochemistry	Metabolism of hydrogen peroxide	9.39E-05	PARK7, PRDX5, SOD1	3
Cellular Function and Maintenance	Homeostasis of muscle cells	9.99E-05	ALDOA, SOD1	2
Lipid Metabolism, Small Molecule Biochemistry	Synthesis of fatty acid	1.38E-04	ACADVL, PARK7, PKM, SOD1	4
Free Radical Scavenging, Small Molecule Biochemistry	Degradation of hydrogen peroxide	1.40E-04	PRDX5, SOD1	2
Carbohydrate Metabolism, Energy Production	Metabolism of pyruvic acid	1.75E-04	ALDOA, PKM	2
Cellular Function and Maintenance	Respiration of mitochondria	3.35E-04	PKM, SOD1	2
Cell Morphology, Cellular Function and Maintenance	Mitochondrial membrane potential	3.70E-04	PARK7, SOD1	2
Cellular Assembly and Organization	Binding of zona pellucida	3.87E-04	ALDOA, ZBP2	2
Drug Metabolism, Protein Synthesis	Metabolism of glutathione	4.63E-04	GSTM3, SOD1	2
Cell-To-Cell Signaling and Interaction, Reproductive System Development and Function	Binding of sperm	5.67E-04	ALDOA, ZBP2	2
Cell Morphology, Cellular Function and Maintenance	Transmembrane potential of mitochondria	7.43E-04	HSPD1, PARK7, SOD1	3
Drug Metabolism	Metabolism of xenobiotic	1.05E-03	GSTM3, GSTO2	2
Amino Acid Metabolism, Small Molecule	Synthesis of amino acids	1.27E-03	PARK7, PKM	2

Biochemistry					
Organismal Injury and Abnormalities	Oxidative stress	1.51E-03	PRDX5, SOD1		2
Cell Death and Survival	Cell death of nervous tissue cell lines	1.65E-03	PARK7, SOD1		2
Cell Signaling, Small Molecule Biochemistry	Synthesis of nitric oxide	1.69E-03	HSPD1, PARK7, SOD1		3
Drug Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of glutathione	1.96E-03	PKM, SOD1		2
Cell Death and Survival, Organismal Injury and Abnormalities	Cell death of epithelial cell lines	1.96E-03	HSPD1, PARK7, SOD1		3
Carbohydrate Metabolism, Small Molecule Biochemistry	Metabolism of D-hexose	2.24E-03	ALDOA, PKM		2
Cell Death and Survival	Cell viability of brain cells	2.60E-03	PARK7, SOD1		2
Cell Morphology, Cellular Assembly and Organization	Abnormal morphology of Nucleus	2.64E-03	SOD1, ZBP2		2
Cell-To-Cell Signaling and Interaction, Drug Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of dopamine	2.92E-03	PARK7, SOD1		2
Cellular Assembly and Organization, Cellular Function and Maintenance	Organization of cytoplasm	3.35E-03	ALDOA, EFHD1, KRT6A, PARK7, PKM, SOD1		6
Behavior	Locomotion	3.41E-03	ACADVL, PARK7, SOD1		3
Cell Signaling	Activation of enzyme	3.75E-03	HSPD1, PARK7, SOD1		3
Carbohydrate Metabolism	Glycolysis of cells	4.66E-03	ALDOA, PKM		2
Energy Production, Molecular Transport, Nucleic Acid Metabolism, Small Molecule Biochemistry	Concentration of ATP	4.84E-03	ALDOA, PKM		2
Cell Morphology, Cellular Assembly and Organization	Morphology of mitochondria	5.15E-03	HSPD1, SOD1		2
Organismal Functions	Energy expenditure	5.53E-03	ACADVL, SOD1		2
Carbohydrate Metabolism	Accumulation of carbohydrate	5.93E-03	PKM, SOD1		2
Embryonic Development, Organismal Development, Reproductive System Development and Function	Fertilization	6.62E-03	PARK7, ZBP2		2
Inflammatory Response	Inflammatory response	6.82E-03	HSPD1, PARK7, PRDX5, SOD1		4
Cell Death and Survival	Apoptosis of neurons	7.24E-03	HSPD1, PARK7, SOD1		3
Cell Death and Survival	Cell viability of carcinoma cell lines	7.80E-03	PARK7, PKM		2

Cellular Compromise, Cellular Function and Maintenance	Endoplasmic reticulum stress response	8.10E-03	HSPD1, SOD1	2
Inflammatory Response, Organismal Injury and Abnormalities	Inflammation of organ	8.26E-03	ACADVL, ALDOA, KRT6A, PKM, SOD1	5
Cell Death and Survival	Cell death of hepatoma cell lines	9.72E-03	PKM, SOD1	2
Organ Morphology, Skeletal and Muscular System Development and Function	Morphology of skeletal muscle	1.08E-02	ACADVL, SOD1	2
Cellular Movement	Migration of cells	1.08E-02	ALDOA, HSPD1, PARK7, PKM, SOD1, TUBB2B	6
Lipid Metabolism, Small Molecule Biochemistry	Homeostasis of lipid	1.08E-02	ACADVL, SOD1	2
Protein Degradation, Protein Synthesis	Stabilization of protein	1.10E-02	HSPD1, PARK7	2
Cell Death and Survival	Cell death of neuroblastoma cell lines	1.11E-02	PARK7, SOD1	2
Skeletal and Muscular System Development and Function	Morphology of muscle	1.19E-02	ACADVL, PARK7, SOD1	3
Cellular Assembly and Organization, Cellular Function and Maintenance	Organization of cytoskeleton	1.20E-02	ALDOA, EFHD1, KRT6A, PKM, SOD1	5
Carbohydrate Metabolism	Quantity of carbohydrate	1.32E-02	ACADVL, PKM, SOD1	3
DNA Replication, Recombination, and Repair	DNA damage	1.41E-02	PARK7, SOD1	2
Cellular Assembly and Organization, Cellular Function and Maintenance	Organization of filaments	1.47E-02	ALDOA, SOD1	2
Cellular Assembly and Organization	Organization of organelle	1.61E-02	ALDOA, PARK7, SOD1	3
Cell Death and Survival	Cell death of connective tissue cells	1.73E-02	HSPD1, PARK7, SOD1	3
Cell-To-Cell Signaling and Interaction, Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response	Activation of macrophages	1.77E-02	HSPD1, SOD1	2
Cell Death and Survival, Nervous System Development and Function	Cell viability of neurons	1.77E-02	PARK7, SOD1	2
Skeletal and Muscular System Development and Function	Muscle contraction	1.84E-02	ALDOA, SOD1	2
Protein Degradation, Protein Synthesis	Catabolism of protein	1.92E-02	HSPD1, PARK7, SOD1	3
Cellular Function and Maintenance	Cellular homeostasis	1.92E-02	ALDOA, HSPD1, PARK7, PKM, SOD1	5
Carbohydrate Metabolism	Metabolism of carbohydrate	1.94E-02	ALDOA, GSTO2, PKM	3
Cell Signaling, Post-Translational Modification	Activation of Protein	1.98E-02	PARK7, SOD1	2

	kinase			
Cell Death and Survival	Cytolysis	2.19E-02	ALDOA, KRT6A	2
Cell Morphology, Organismal Injury and Abnormalities	Hypertrophy of cells	2.59E-02	PARK7, SOD1	2
Cell Morphology, Cellular Function and Maintenance	Autophagy of cells	2.77E-02	PKM, SOD1	2
Cell Death and Survival	Apoptosis of tumor cell lines	3.19E-02	HSPD1, PARK7, PKM, SOD1	4
Cellular Growth and Proliferation	Colony formation of tumor cell lines	3.21E-02	PKM, SOD1	2
Nervous System Development and Function	Sensation	3.47E-02	PKM, SOD1	2
Cell Death and Survival	Cell death of carcinoma cell lines	3.68E-02	PKM, SOD1	2
Cell Death and Survival	Apoptosis	4.15E-02	ALDOA, HSPD1, PARK7, PKM, PRDX5, SOD1	6
Reproductive System Development and Function	Fertility	4.17E-02	SOD1, ZPBP2	2
Free Radical Scavenging	Production of reactive oxygen species	4.25E-02	PARK7, SOD1	2
Cell Death and Survival, Embryonic Development	Cell death of embryonic cell lines	4.28E-02	PARK7, SOD1	2
Cell Death and Survival	Cell death	4.37E-02	ALDOA, HSPD1, KRT6A, PARK7, PKM, PRDX5, SOD1	7
Cell Death and Survival	Cell viability	4.82E-02	HSPD1, PARK7, PKM, SOD1	4

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**Table S3.** Molecular and cellular functions performed by proteins changing in adult bulls supernatant of cryopreserved semen in comparison to young bulls supernatant of cryopreserved semen

Categories	Diseases or Functions Annotation	p-Value	Molecules	# Molecules
Cell Morphology	Morphology of leukocytes	2.40E-06	B2M, NGF, NPC2, PTGDS	4
Cell Morphology, Inflammatory Response	Morphology of phagocytes	1.14E-05	NGF, NPC2, PTGDS	3
Cell Morphology, Hematological System Development and Function, Hypersensitivity Response, Inflammatory Response	Morphology of mast cells	1.44E-05	NGF, PTGDS	2
Cardiovascular System Development and Function, Cell Morphology, Cellular Development, Cellular Function and Maintenance, Cellular Growth and Proliferation, Organismal Development, Tissue Development	Morphogenesis of vascular endothelial cells	1.57E-05	NGF, TIMP2	2
Lipid Metabolism, Small Molecule Biochemistry	Synthesis of prostaglandin	1.64E-05	NGF, NPC2, PTGDS	3
Lipid Metabolism, Small Molecule Biochemistry	Synthesis of prostaglandin D2	3.48E-05	NPC2, PTGDS	2
Cell Death and Survival	Apoptosis of oligodendrocytes	5.34E-05	NGF, PTGDS	2
Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response, Tissue Development	Accumulation of leukocytes	7.18E-05	B2M, PTGDS, TIMP2	3
Molecular Transport, Small Molecule Biochemistry	Release of histamine	7.28E-05	B2M, NGF	2
Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response	Migration of phagocytes	8.16E-05	NGF, PTGDS, TIMP2	3
Immunological Disease	Abnormal morphology of immune system	1.19E-04	B2M, NPC2, PTGDS	3
Hematological System Development and Function, Organismal Functions	Flow of blood	1.21E-04	NGF, PTGDS	2
Cell-To-Cell Signaling and Interaction, Drug Metabolism, Molecular Transport, Small Molecule	Release of dopamine	1.58E-04	NGF, TIMP2	2

Biochemistry					
Cell Death and Survival	Cell death of leukemia cell lines	1.58E-04	B2M, NGF, NPC2		3
Organismal Injury and Abnormalities, Tissue Morphology	Size of lesion	1.84E-04	NGF, PTGDS, TIMP2		3
Hematological System Development and Function, Tissue Morphology	Quantity of leukocytes	1.96E-04	B2M, NGF, NPC2, TIMP2		4
DNA Replication, Recombination, and Repair	Synthesis of DNA	2.77E-04	NGF, PTGDS, TIMP2		3
Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response	Migration of monocytes	3.11E-04	NGF, TIMP2		2
Nervous System Development and Function, Tissue Morphology	Quantity of neurons	3.30E-04	B2M, NGF, NPC2		3
Hematological System Development and Function, Inflammatory Response, Tissue Morphology	Quantity of phagocytes	3.68E-04	B2M, NGF, TIMP2		3
Cardiovascular System Development and Function, Tissue Morphology	Permeability of blood vessel	3.82E-04	NGF, TIMP2		2
Cell Morphology, Nervous System Development and Function, Tissue Morphology	Size of neurons	3.82E-04	B2M, NGF		2
Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response, Tissue Development	Accumulation of granulocytes	4.68E-04	PTGDS, TIMP2		2
Cellular Movement	Migration of melanoma cell lines	4.91E-04	NGF, TIMP2		2
Hematological System Development and Function, Tissue Morphology	Quantity of myeloid cells	5.50E-04	B2M, NGF, TIMP2		3
Behavior, Nervous System Development and Function	Circadian rhythm	5.71E-04	NGF, PTGDS		2
Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of eicosanoid	5.71E-04	NGF, PTGDS		2
Organismal Injury and Abnormalities, Tissue Morphology	Volume of lesion	6.22E-04	NGF, TIMP2		2
Cell Morphology	Size of cells	6.66E-04	B2M, NGF, PTGDS		3
Neurological Disease, Organismal Injury and Abnormalities	Degeneration of brain	6.93E-04	NGF, NPC2		2
Cancer, Hematological Disease, Immunological Disease, Organismal Injury and Abnormalities	T-cell non-Hodgkin disease	7.27E-04	B2M, BSPH1, TIMP2		3
Cancer, Hematological Disease, Immunological Disease, Organismal Injury and Abnormalities	T-cell leukemia/lymphoma	7.68E-04	B2M, BSPH1		2



Drug Metabolism, Lipid Metabolism, Small Molecule Biochemistry	Synthesis of prostaglandin E2	7.97E-04	NGF, NPC2	2
Cellular Compromise, Hypersensitivity Response, Inflammatory Response	Degranulation of mast cells	8.16E-04	NGF, PTGDS	2
Neurological Disease	Tremor	9.18E-04	NGF, NPC2	2
Cellular Movement	Cell movement of myeloid cells	9.25E-04	NGF, PTGDS, TIMP2	3
Dermatological Diseases and Conditions, Neurological Disease, Organismal Injury and Abnormalities	Hyperesthesia	9.39E-04	NGF, PTGDS	2
Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response, Tissue Development	Accumulation of phagocytes	9.60E-04	PTGDS, TIMP2	2
Nervous System Development and Function, Tissue Morphology	Quantity of central nervous system cells	9.82E-04	NGF, NPC2	2
Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response, Tissue Development	Accumulation of mononuclear leukocytes	1.03E-03	B2M, PTGDS	2
Cell Death and Survival	Necrosis	1.08E-03	B2M, NGF, NPC2, PTGDS, TIMP2	5
Metabolic Disease	Amyloidosis	1.14E-03	B2M, NGF, PTGDS	3
Cellular Development, Connective Tissue Development and Function	Differentiation of fibroblast cell lines	1.28E-03	NPC2, PTGDS	2
Cell Morphology, Hematological System Development and Function	Morphology of myeloid cells	1.37E-03	NPC2, PTGDS	2
Cellular Movement	Migration of myeloid cells	1.54E-03	PTGDS, TIMP2	2
Cell Signaling, Molecular Transport, Nucleic Acid Metabolism, Small Molecule Biochemistry	Concentration of cyclic AMP	1.58E-03	NGF, TIMP2	2
Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	Secretion of lipid	1.61E-03	NGF, NPC2	2
Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of phospholipid	1.87E-03	NGF, NPC2	2
Hematological System Development and Function, Lymphoid Tissue Structure and Development, Tissue Morphology	Quantity of lymphocytes	1.92E-03	B2M, NGF, NPC2	3
Cell Death and Survival, Cellular Compromise	Cytotoxicity of cells	2.14E-03	B2M, NGF	2
Cell Morphology, Cellular Function and Maintenance	Transmembrane potential of mitochondria	2.17E-03	B2M, NGF	2

Cellular Movement	Invasion of carcinoma cell lines	2.62E-03	NGF, TIMP2	2
Nucleic Acid Metabolism, Small Molecule Biochemistry	Metabolism of cyclic nucleotides	2.80E-03	NGF, TIMP2	2
Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry	Concentration of lipid	2.83E-03	NGF, NPC2, PTGDS	3
Cell Death and Survival, Cellular Compromise, Neurological Disease, Tissue Morphology	Degeneration of neurons	2.86E-03	NGF, NPC2	2
Cellular Function and Maintenance, Hematological System Development and Function	Function of T lymphocytes	3.06E-03	B2M, NPC2	2
Nutritional Disease	Weight loss	3.08E-03	NGF, NPC2	2
Cancer, Hematological Disease, Immunological Disease, Organismal Injury and Abnormalities	Multiple myeloma	3.41E-03	B2M, TIMP2	2
Cell-To-Cell Signaling and Interaction, Nervous System Development and Function	Long-term potentiation	3.48E-03	B2M, NGF	2
Cell Cycle	Arrest in G1 phase	3.54E-03	NGF, TIMP2	2
Behavior	Emotional behavior	3.81E-03	B2M, NGF	2
Embryonic Development, Organismal Development, Tissue Development	Growth of embryonic tissue	3.81E-03	NGF, TIMP2	2
Cell Death and Survival	Apoptosis of leukemia cell lines	3.90E-03	B2M, NGF	2
Cell Death and Survival	Apoptosis of breast cancer cell lines	4.53E-03	B2M, NGF	2
Cell-To-Cell Signaling and Interaction	Activation of cells	4.71E-03	B2M, NGF, NPC2	3
Cellular Growth and Proliferation	Expansion of cells	4.88E-03	B2M, NGF	2
Organismal Injury and Abnormalities	Discomfort	4.92E-03	NGF, TIMP2	2
Immunological Disease, Inflammatory Disease, Inflammatory Response, Neurological Disease, Organismal Injury and Abnormalities	Experimental autoimmune encephalomyelitis	5.18E-03	B2M, NGF	2
Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism	Mobilization of Ca <sup>2+</sup>	5.70E-03	B2M, NGF	2
Cardiovascular Disease, Organismal Injury and Abnormalities	Infarction	6.46E-03	NGF, PTGDS	2
Cardiovascular System Development and Function	Development of vasculature	6.73E-03	NGF, NPC2, TIMP2	3
Embryonic Development, Organismal Development	Development of body trunk	7.16E-03	B2M, NGF, NPC2	3
Cardiovascular System Development and Function	Morphology of	7.48E-03	NPC2, TIMP2	2

	vasculature			
Cell Morphology, Cellular Development	Branching of cells	8.43E-03	NGF, TIMP2	2
Organismal Survival	Organismal death	8.93E-03	B2M, NGF, NPC2, TIMP2	4
Immunological Disease	Hypersensitive reaction	9.65E-03	NGF, PTGDS	2
Cellular Development, Nervous System	Differentiation of neurons	9.91E-03	NGF, TIMP2	2
Development and Function, Tissue Development				
Cell Death and Survival	Apoptosis of neurons	1.04E-02	NGF, PTGDS	2
Nervous System Development and Function	Development of Visual system	1.14E-02	NGF, TIMP2	2
Cell Death and Survival	Apoptosis	1.22E-02	B2M, NGF, PTGDS, TIMP2	4
Infectious Diseases	Replication of RNA virus	1.26E-02	B2M, NGF	2
Cellular Development, Cellular Growth and Proliferation	Cell proliferation of breast cancer cell lines	1.28E-02	NGF, TIMP2	2
Molecular Transport	Quantity of metal	1.29E-02	B2M, NGF	2
Cell Cycle	Mitosis	1.36E-02	NGF, TIMP2	2
Cell Morphology, Nervous System Development and Function, Neurological Disease, Tissue Morphology	Abnormal morphology of neurons	1.38E-02	B2M, NGF	2

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