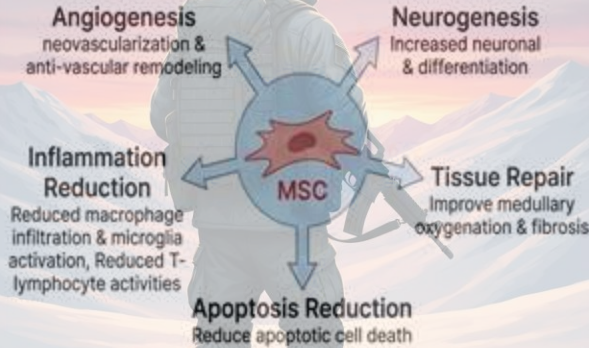


**Mesenchymal Stem Cell (MSC)
Therapeutics**



Exosomes derived from human MSCs restore bioenergy and tissue repair. Crucial benefit: Easy to store and handle in austere environments.

Exosomes Derived from Human Stem Cells Acts as Early Stabilizer to Ameliorate Symptoms of Cold Stress: Systems Biology Analysis

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Disclaimers

The views expressed in this presentation reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Defense Health Agency, Department of War, nor the U.S. Government.

The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the “Guide for Care and Use of Laboratory Animals,” Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

No endorsement of non-federal entity, product, service, or activity is approved or implied by this presentation

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Core Capabilities & Strategic Ecosystem

Deep Discovery

Next-Gen Sequencing



Single Cell Omics



Spectral Assays (LC/MS, OLINK)



Automated Liquid Handling



Advanced Models

3D Cell Culture



Gnotobiotic Vivarium



Space Biology/ISS



Data Maneuvering

DoD HPC Access



Custom Scripts/Libraries

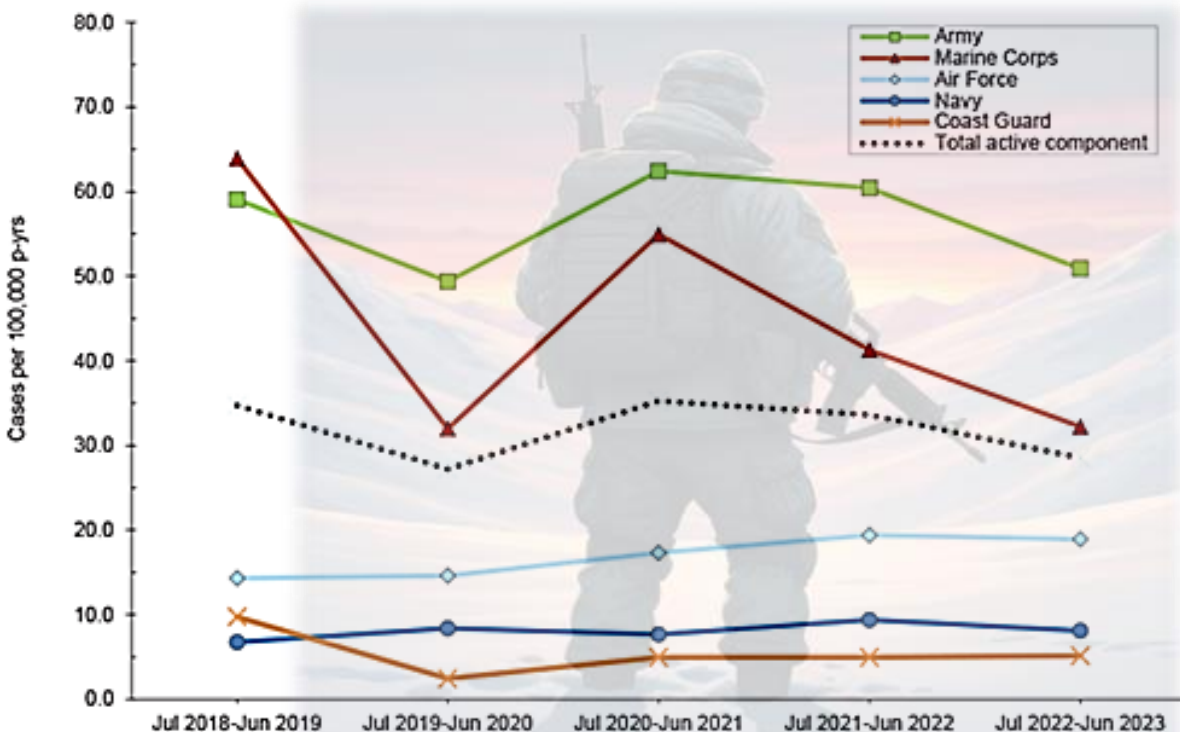


Big Data Management



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Growing number of deployment in extreme cold condition

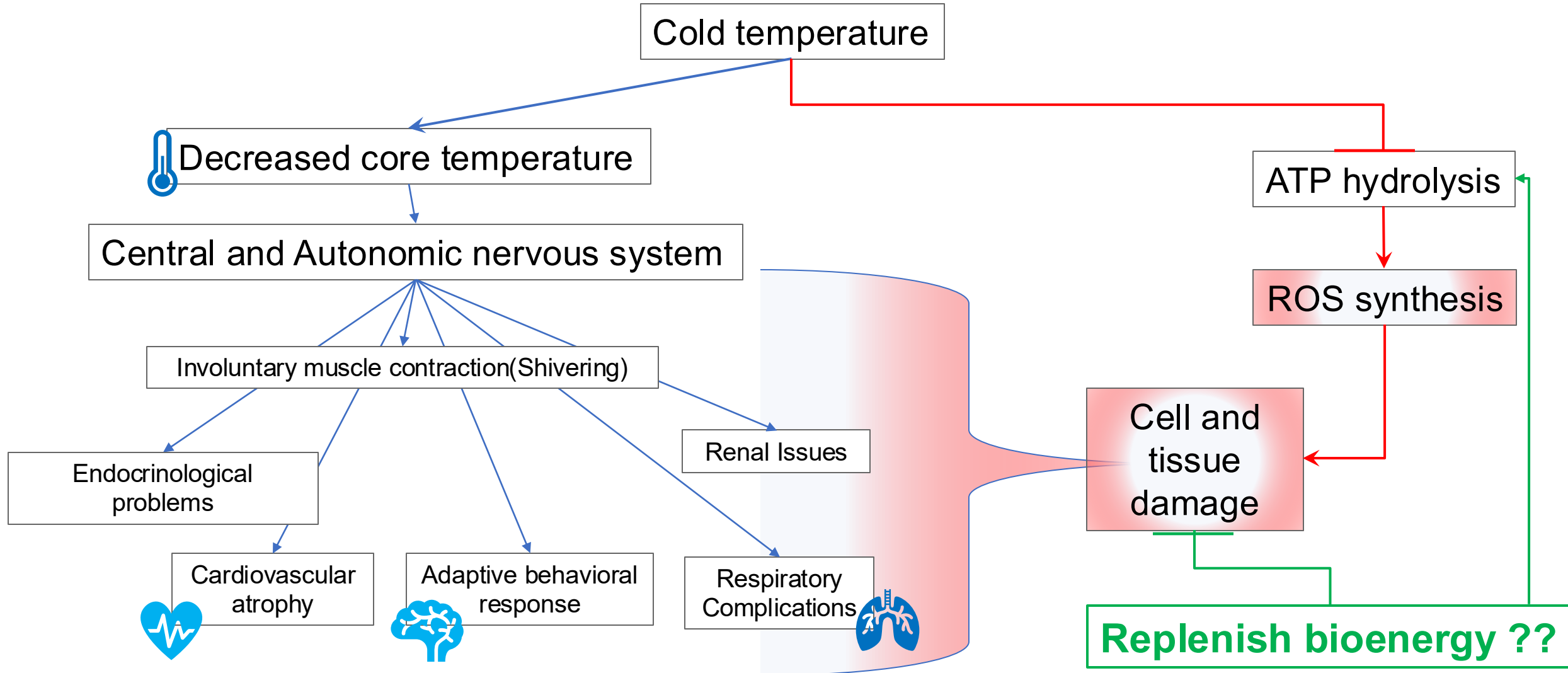


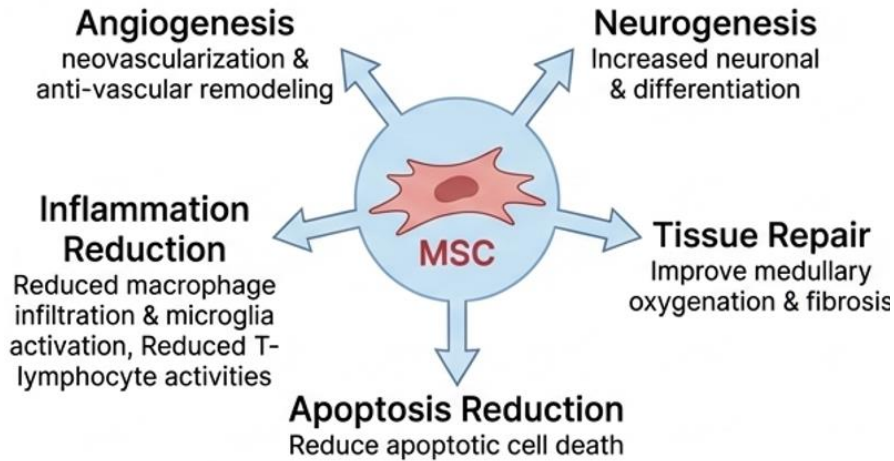
Worldwide, >1k soldiers regularly deployed or trained in these geopolitically crucial regions-

- Alaska and arctic edge
- NATO arctic terrain (Norway)
- Siachen glacier and Himalayan range

Longitudinal data showing number of cold stress related incidences in various DoW units.

Reference: Armed Forces Health Surveillance, D. Cold Weather Injuries Among the Active and Reserve Components of the U.S. Armed Forces, July 2018-June 2023. *MSMR* 30, 2-11 (2023).





MSC demonstrated its capability to boost bioenergy, hence a potential countermeasure of cold stress.

Limitations-

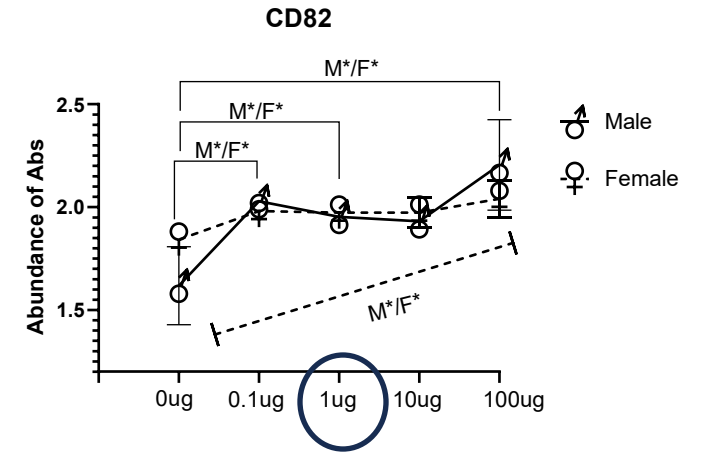
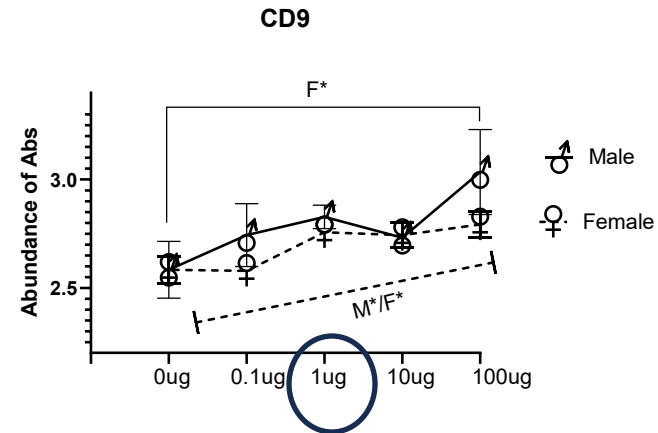
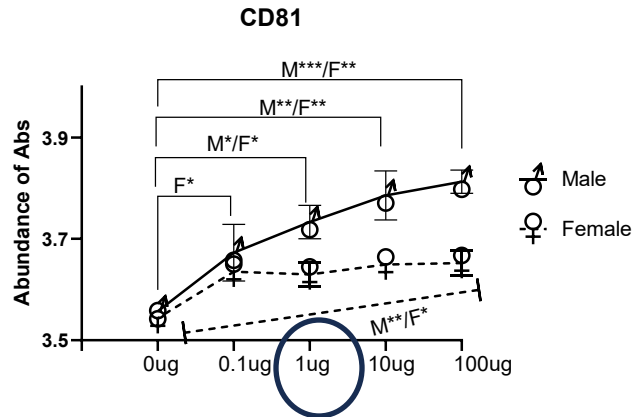
- High batch-to-batch variability in production
- Cold chain essential and limited shelf-life of cells
- Ethical issues

Solution:

Exosomes derived from human MSC (hMSC-ex)

- Showed MSC-like efficacy
- Less batch-to-batch variability in production
- Less complicated archiving protocol

Estimation of bio-availability of exosomes in brain



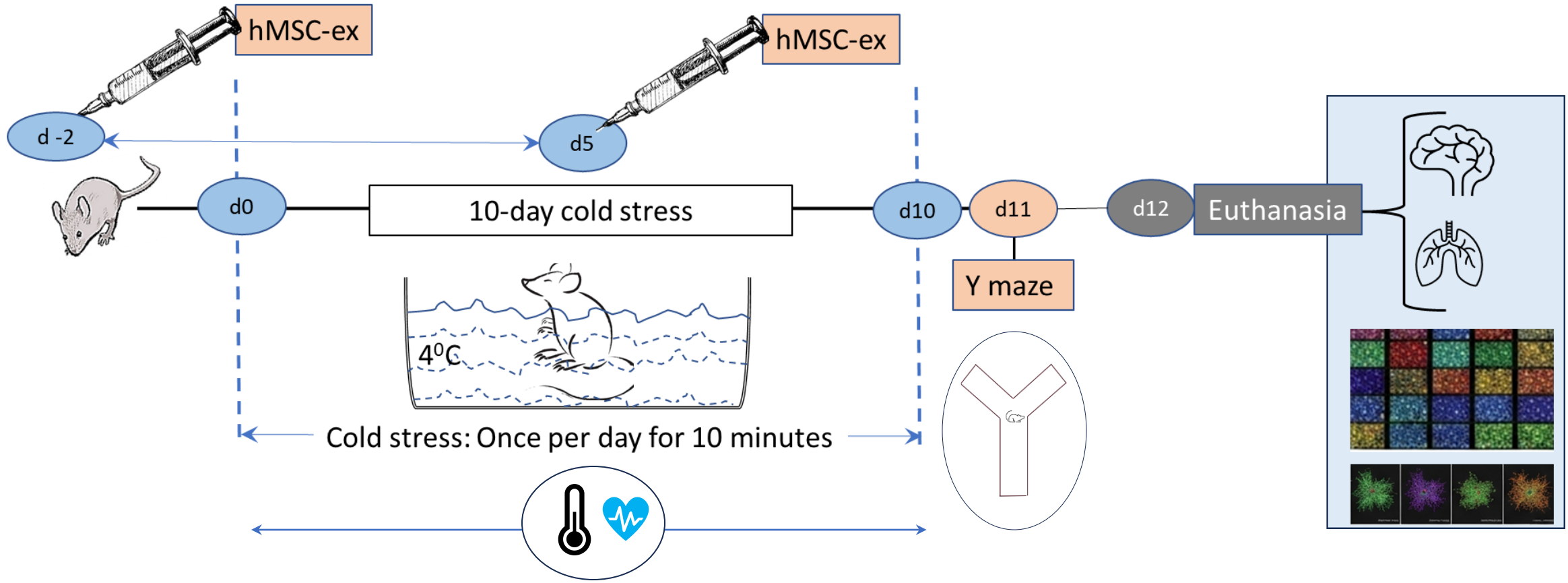
hMSC-ex was purchased from RoosterBio, Inc., MD

Blood inflammation profile:

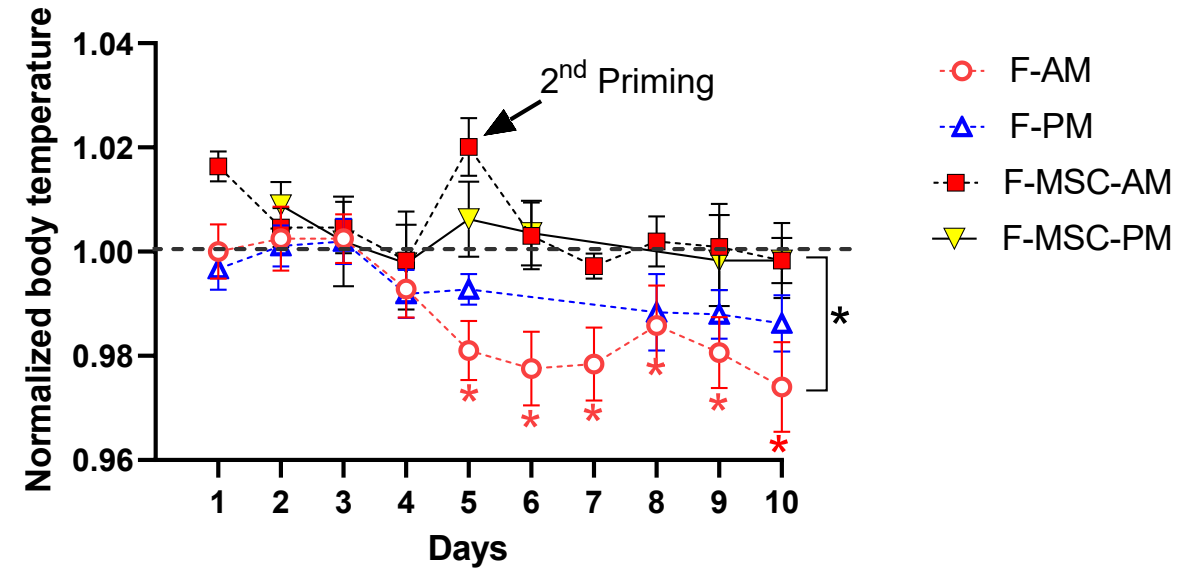
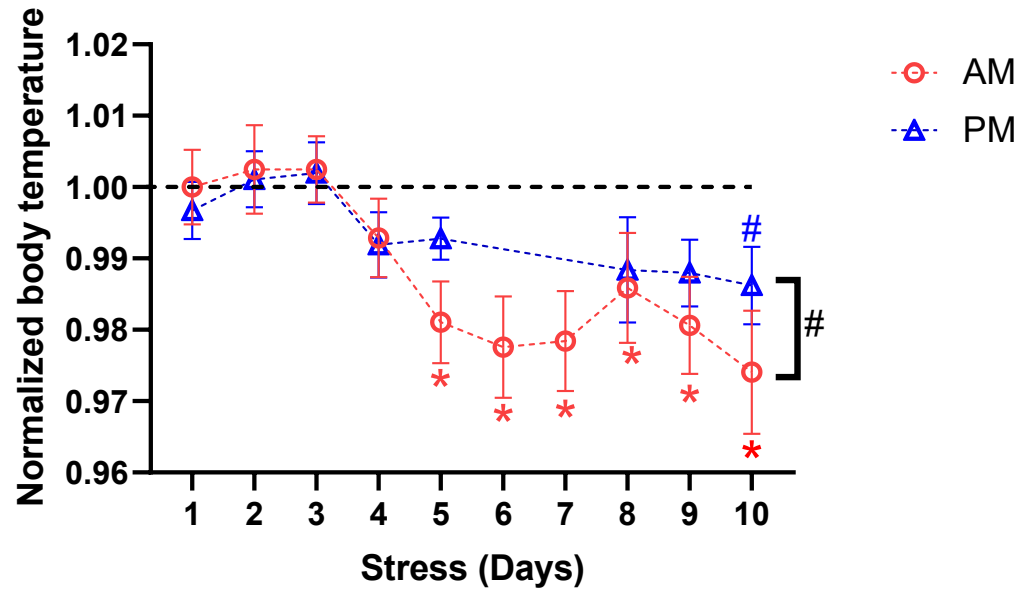
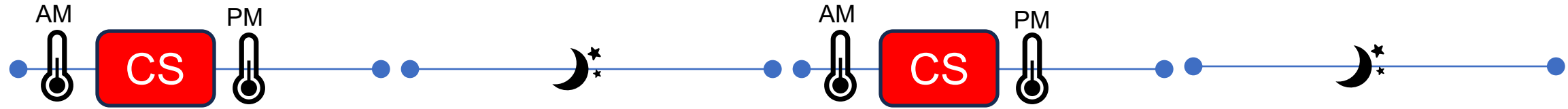
100ug/1.7e10 EV			10ug/1.7e9 EV			1ug/1.7e8 EV		0.1ug/1.7e7 EV	
Protein	logFC	Inflammatory	Protein	logFC	Inflammatory	Protein	logFC	Protein	logFC
Cyr61	1.13	Pro	Cyr61	1.32	Pro	None		None	
Tnfsf12	1.00	Pro	Tnfsf12	1.20	Pro				
Adam23	-1.02		Fli1	1.11	Pro				
Nadk	-1.07	Anti	Ccl2	1.05	Pro				
Itgb1bp2	-1.30		Ntf3	1.00					
			Gcg	-1.05	Anti				
			Nadk	-1.14	Anti				
			Itgb1bp2	-1.28					

Taking all in consideration, we decided to dose 1ug/1.7e8 EV in 1 week

Study design: Sample size 6 per group

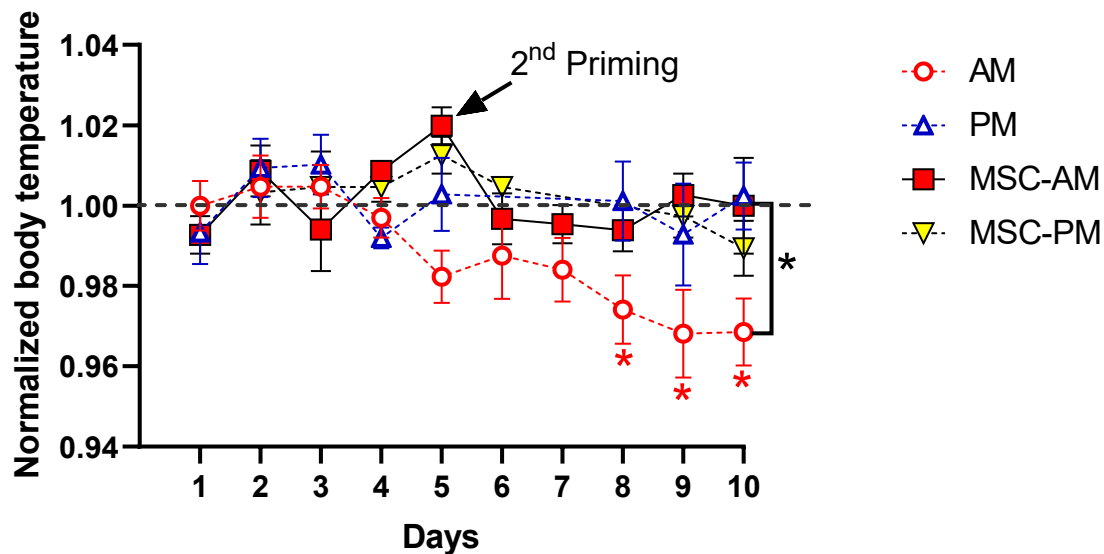
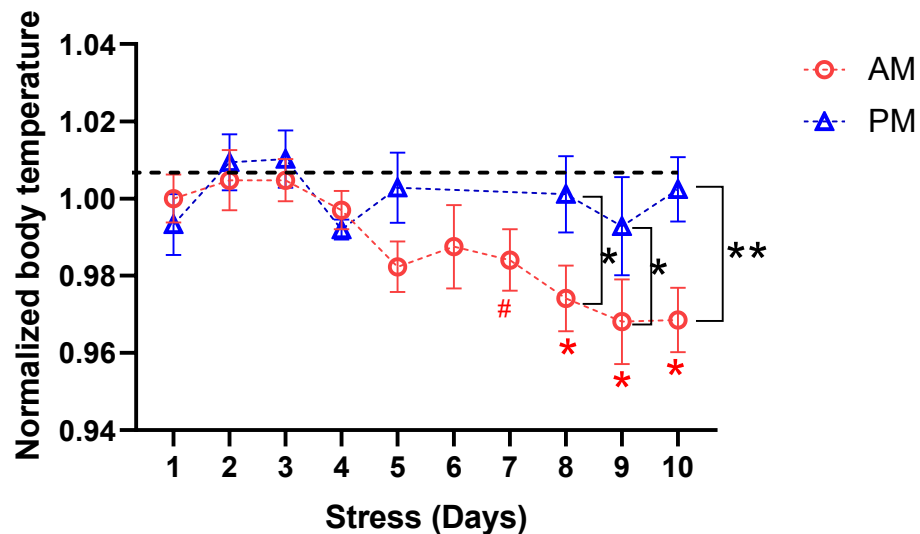
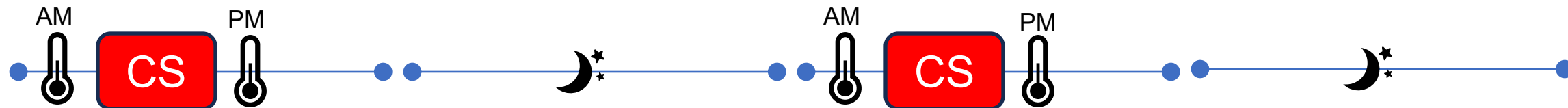


Female: Body temperature

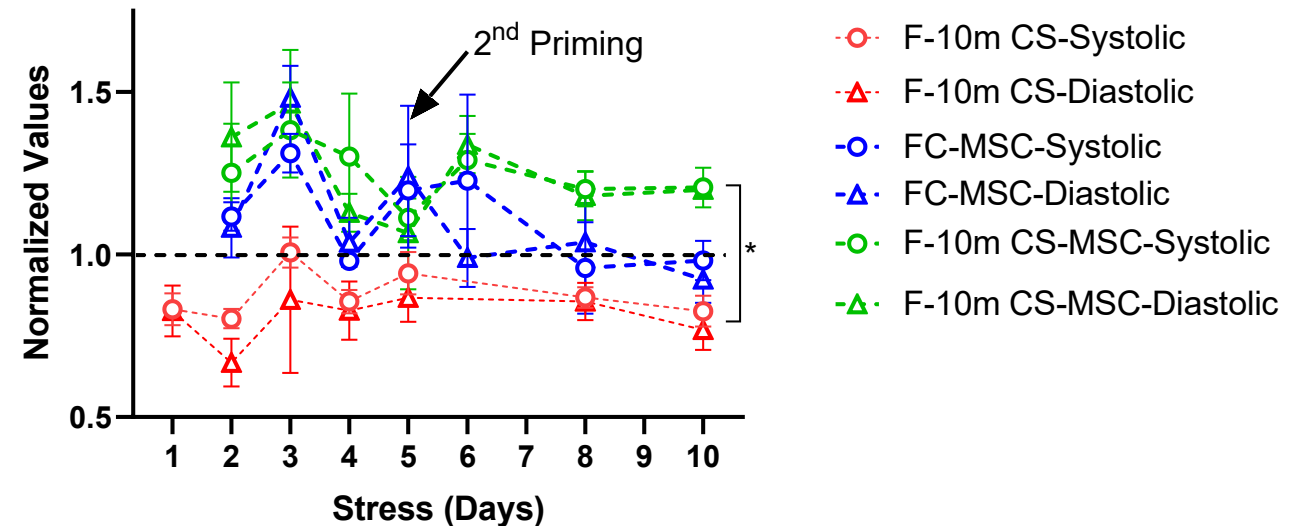
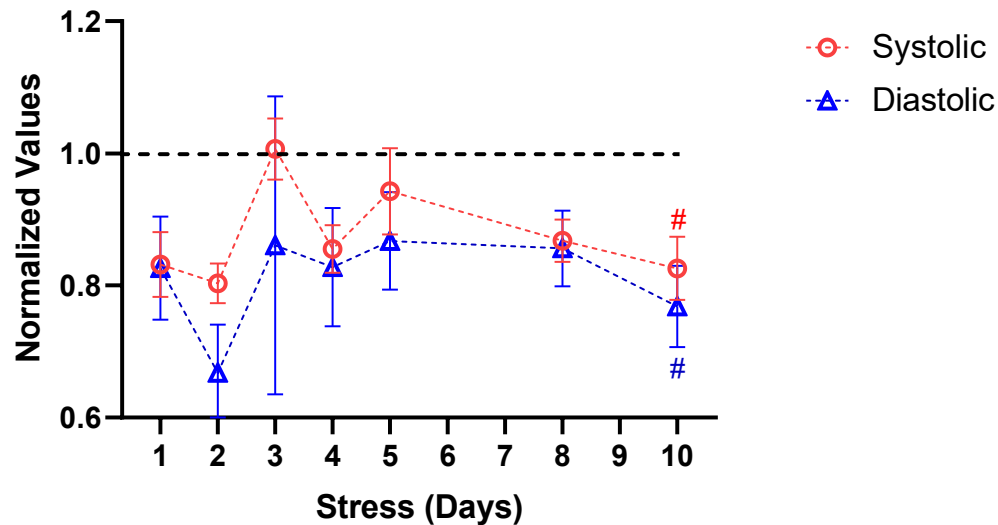


- Female body temperatures measured at AM and PM were significantly reduced from the baseline.
- A consistent thermoneutrality was observed after hMSC-ex administration.

Male: Body temperature

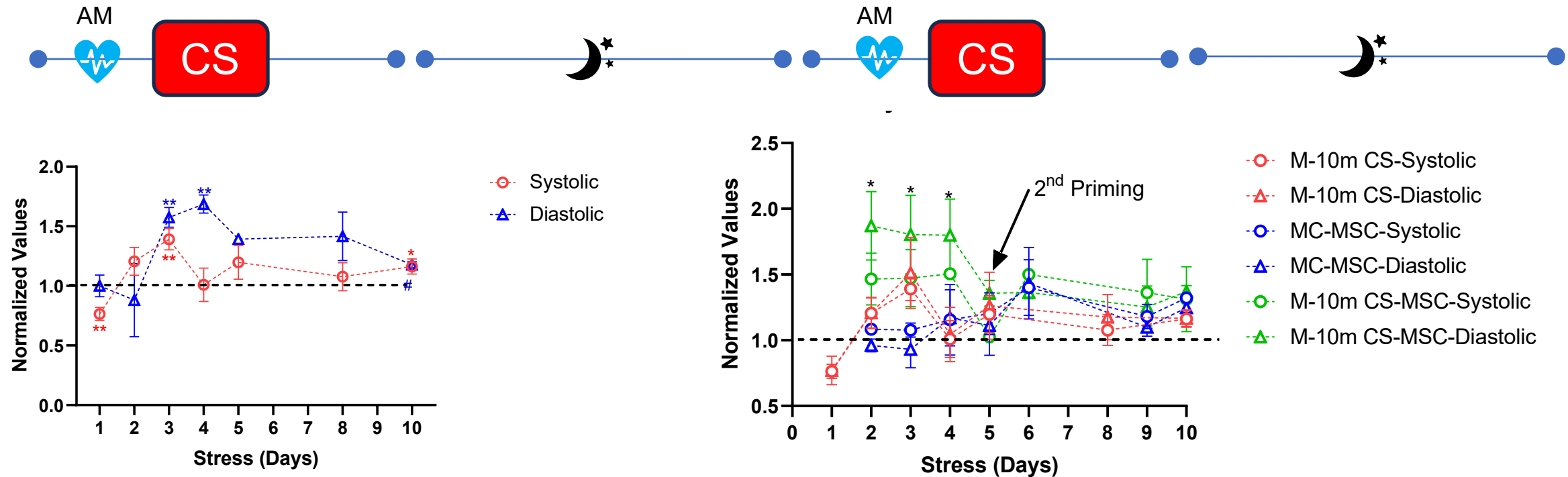


- Male body temperature measured at AM was significantly reduced from the baseline, not the PM temperature.
- A consistent thermoneutrality was observed after hMSC-ex administration.



- Cold stress reduced the female BP from the baseline.
- Administration of hMSC-ex to healthy sham female mice did not cause much changes.
- Administration of hMSC-ex during the cold stress elevated BP.

Male: Blood pressure

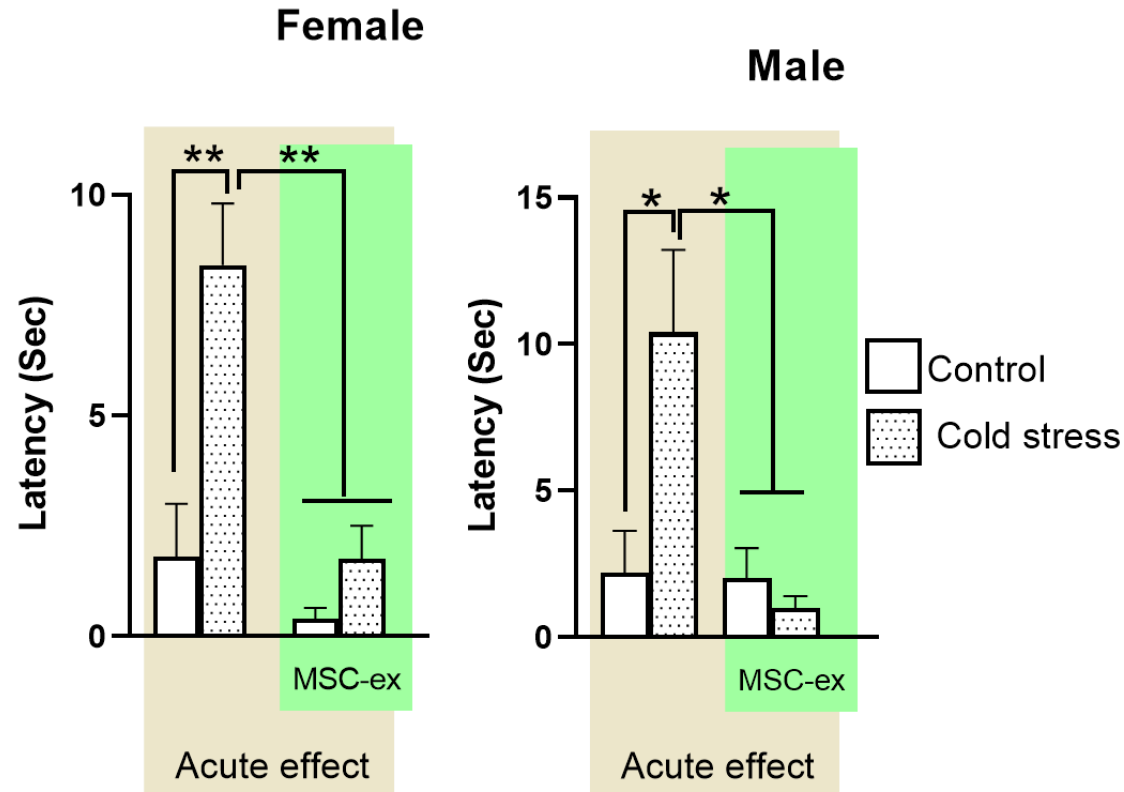
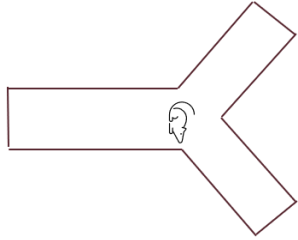


- Cold stress elevated the BP of male mice from the baseline.
- Administration of hMSC-ex to healthy sham male mice did not cause much changes.
- Administration of hMSC-ex during the cold stress elevated the blood pressure significantly at the early days. but moved it to baseline at around day 5 after starting the cold stress regime.

	Cold stress + No MSC-ex		No stress control + hMSC-ex		Cold stress+ hMSC-ex	
	Female	Male	Female	Male	Female	Male
Degeneration, vacuolar, cardiomyocyte	1.8 ± 0.5	2.2 ± 0.5	0.2 ± 0.4	0.25 ± 0.5	1.0 ± 0.7	1.0 ± 0.0

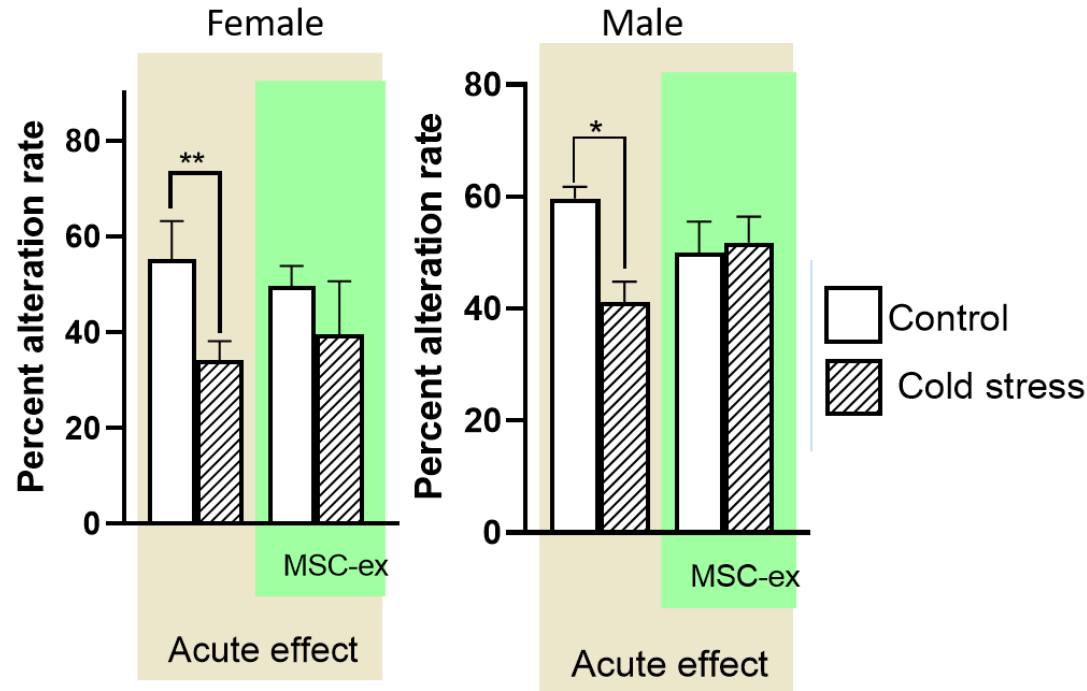
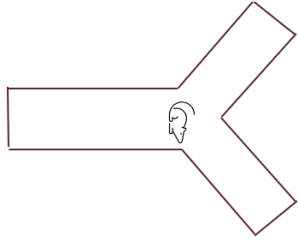
- Cardiac degeneration markers showed reduction in cold stressed mice after hMSC-ex administration.

Y maze: Latency to make first move



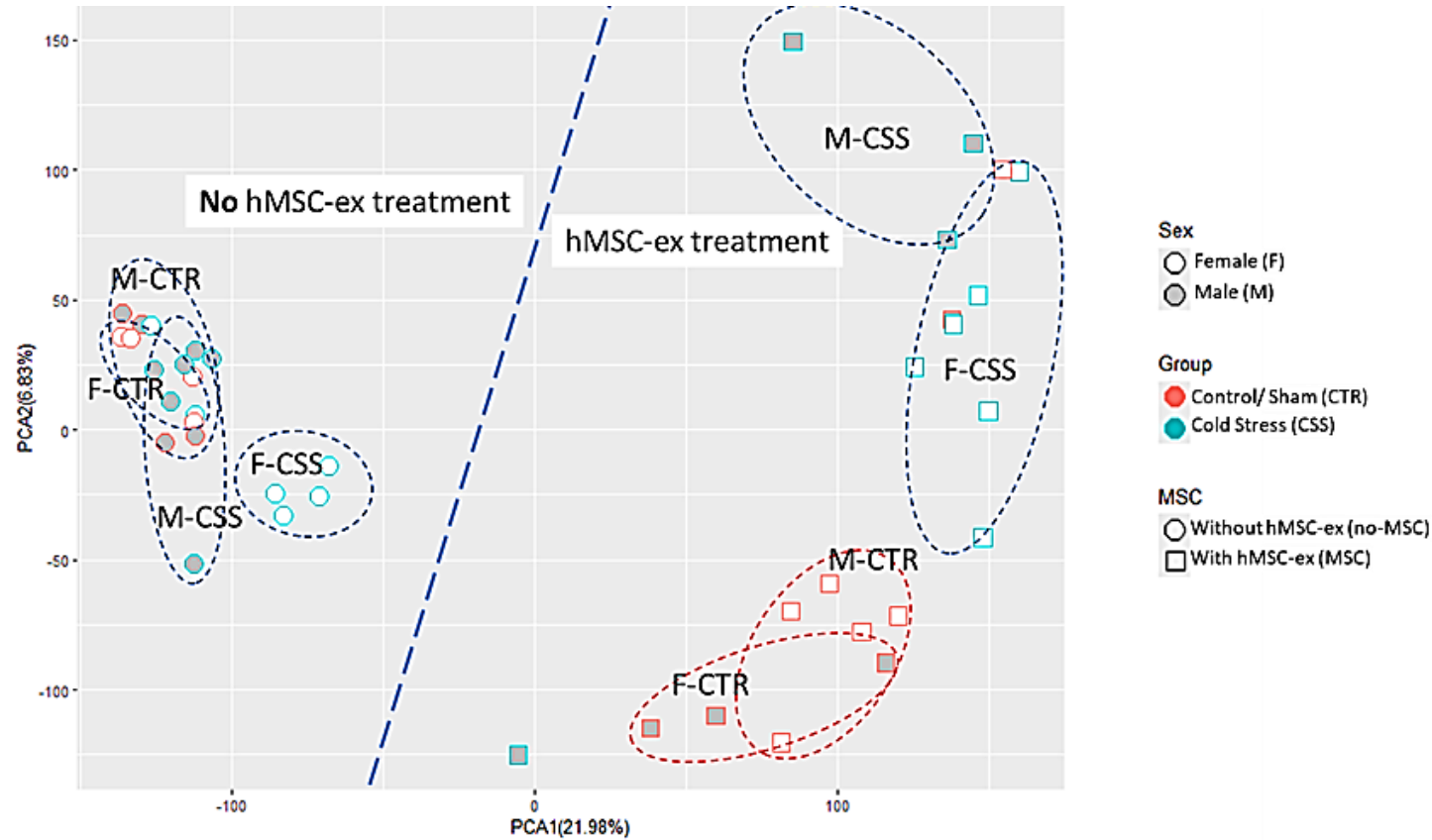
- High latency typically correlates with stress level.
- For both male and female, the latency time was significantly high in cold stressed mice from their controls.
- hMSC-ex administration significantly reduced the latency period.

Y maze: Rate of altering the arms in Y maze



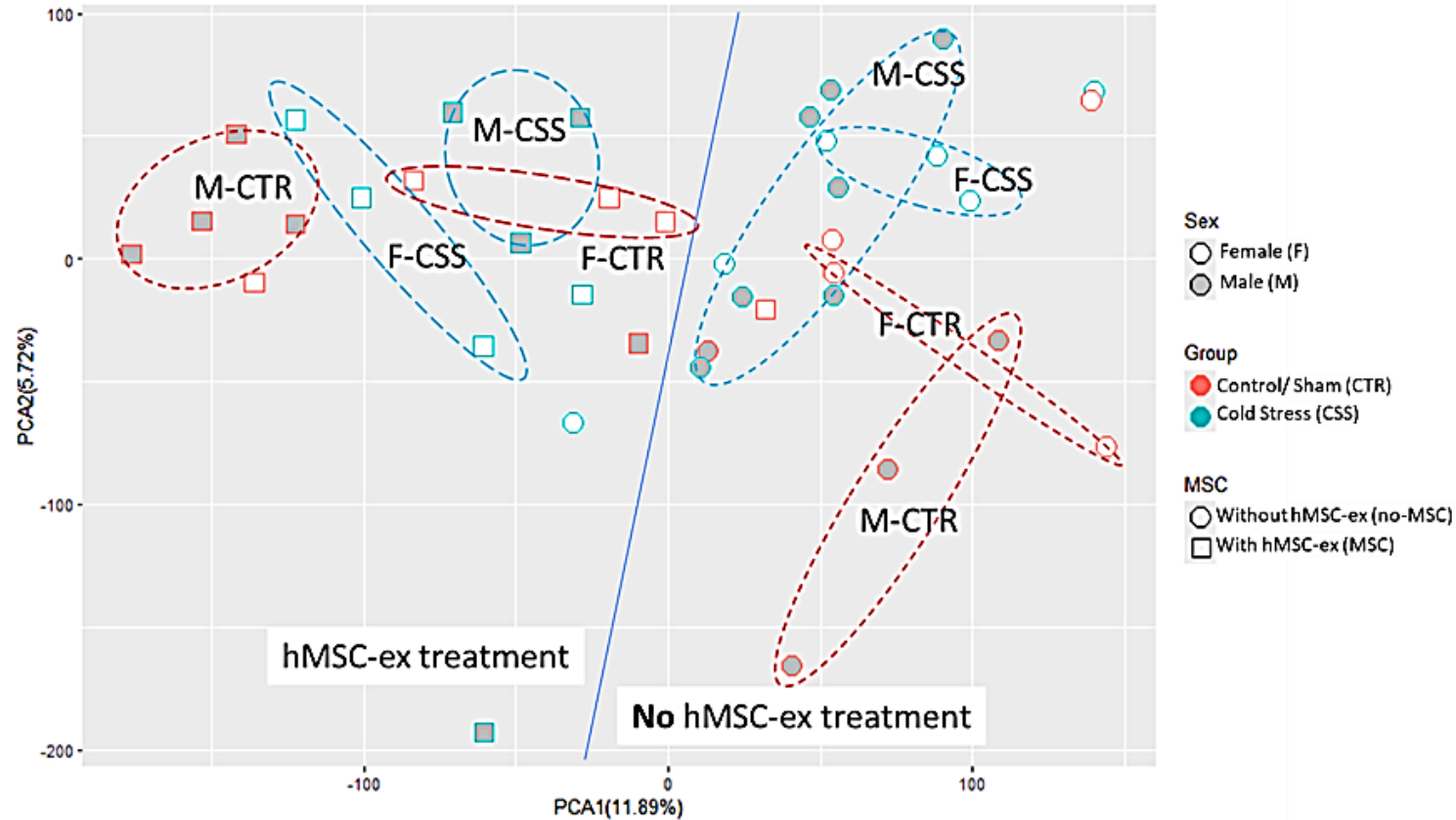
- High rate of altering arms typically represents the natural curious behavior of mice.
- Cold stressed mice displayed significantly low alteration rate.
- Administration of hMSC-ex increased the alteration rate among the stressed mice.

Lungs gene profile: Principal component analysis



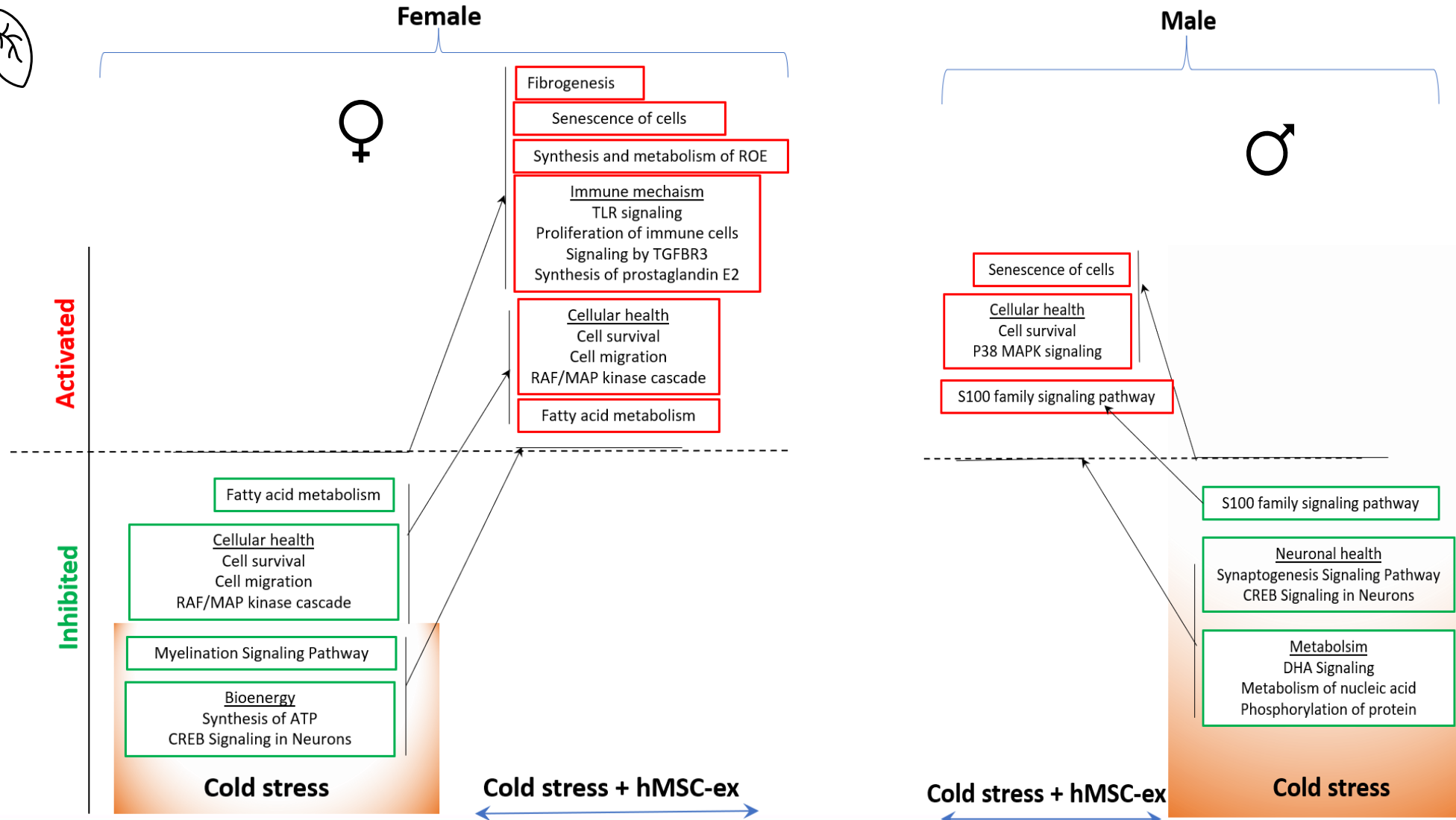
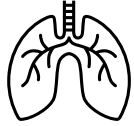
- hMSC-ex treatment caused the largest differences in the lung mRNA landscape.
- Female mice showed the most significant changes caused by cold stress.
- Post-treatment, cold-stressed mice showed a major shift from the control group.

Hemibrain gene profile: Principal component analysis

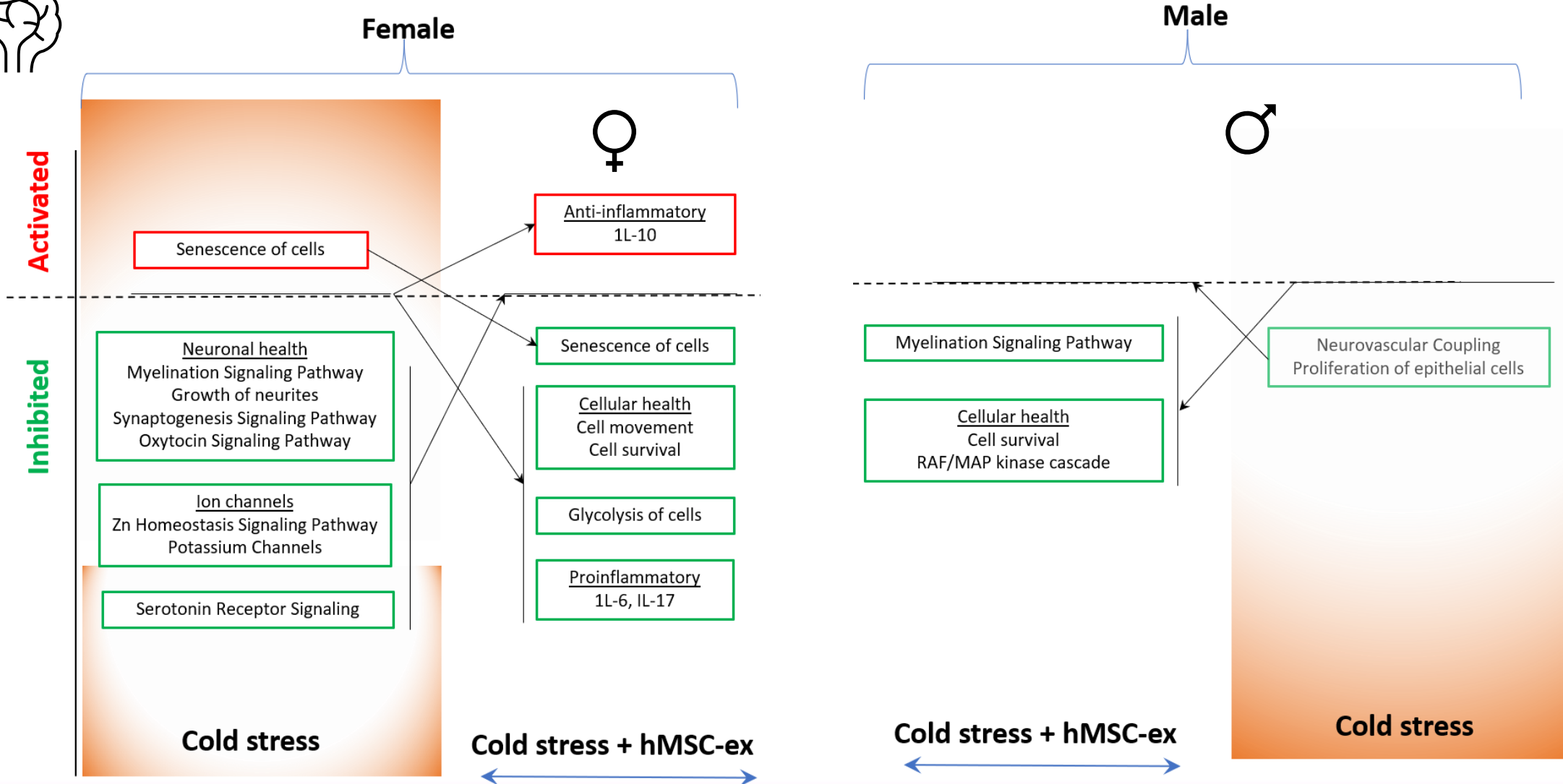


- Like the lungs, the hemibrain showed the greatest mRNA perturbations caused by hMSC-ex treatment.
- The overall profile showed a similar pattern, *although* the degree of separation between the clusters was lesser in the hemibrain than in the lungs.

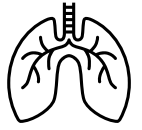
Lungs: Gene networks analysis



Hemibrain: Gene networks analysis



Cell survival gene network: Impact of hMSC-ex

Tissue/ sex		Cell survival	Sub-networks (Canonical)	Sub-networks (Biofunctions)
	♀ Brain/ Female (41 genes)	Inhibited	Serotonin receptor signaling	Apoptosis Neuronal cell death
	♂ Brain/ Male (41 genes)	Inhibited	Serotonin receptor signaling IL5 signaling NF-kb signaling Myelination signaling pathway RAF/MAP kinase pathway	Apoptosis Neuronal cell death
	♀ Lungs/ Female (141 genes)	Activated	AMPK signaling ERK/MPK signaling IL-27 signaling Cachexia signaling pathway TGFR4 signaling	Apoptosis Lung injury
	♂ Lungs/ Male (93 genes)	Activated	IL-15 signaling	Apoptosis Lung injury

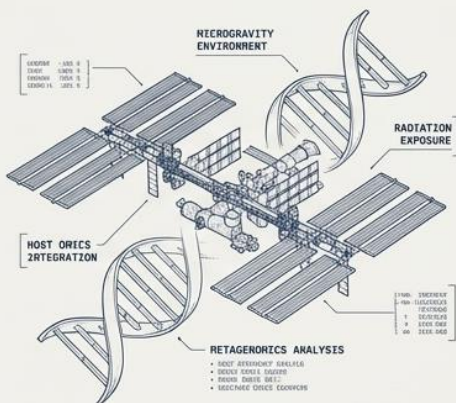
Activated
↑
↓
Inhibited

- A cold stress model was developed to show that 10 days of stress can elicit an optimal level of stress. Five days of stress failed to do so (data not shown).
- The hMSC-ex dose-response profile and literature review determined the dose and administration schedule.
- Behavioral study (Y-maze) showed significant latency as the acute impact of cold stress. **hMSC-ex was able to reduce the latency as well.**
- A significant decrease in core body temperature was observed in both male and female cohorts, although there was a sex bias. **hMSC-ex was able to restore thermoneutrality.**
- Systolic and diastolic blood pressure changed due to cold stress; a sex bias was observed. **hMSC-ex was able to restore normalcy to some extent.**
- Histology of heart tissue showed mild cardiomyocyte degeneration. **hMSC-ex was able to restore normalcy to some extent.**

Conclusions (Continued)

- Omics analysis found that hMSC-ex treatment was a more dominant factor causing perturbations than cold stress.
 - A potential adjustment of dose might be needed.
 - Future studies should focus on the long-term effects of hMSC-ex.
 - Major and positive changes were reported in cold-stressed lung samples after hMSC-ex treatment.
 - *However*, the impact of hMSC-ex on the brain was not adequate, potentially suggesting a limitation of time-sensitive assays.
-
- ❖ The capability of hMSC-ex to ameliorate Acute Stress Response (ASR) has been demonstrated in an additional study.
 - ❖ Disorders associated with acute energy depletion could potentially be mitigated by hMSC-ex.
 - ❖ **hMSC-ex may be deployed as an early stabilizer to counter multiple military-relevant disorders.**

Space & Microgravity

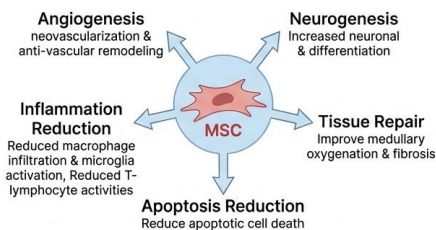


Review of our five Space studies

Session: Current Challenges in DoW Aerospace Medicine

Time: Monday, 1300-1500

Mesenchymal Stem Cell (MSC) Therapeutics



Exosomes derived from human MSCs restore bioenergy and tissue repair. Crucial benefit: Easy to store and handle in austere environments.

hMSC-ex in Cold stress

Session: Human Performance Optimization in Cold-Weather & High-Altitude Environments

Time: Monday, 1530-1730

hMSC-ex in ASD

Session: Cellular Therapeutics for Trauma

Time: Thursday, 1115-1315



TBI Biomarker

Session: Managing the Golden Hour & Beyond: Innovative TBI Care in Contested Combat Environments

Time: Wednesday, 0830-1000

Radiation Therapeutics

Session: Ops in a Nuclear Environment: Ensuring Medical Readiness & Preparing for Complex Injuries

Time: Wednesday, 1530:1730

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