On Orbit Sample Freezing

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October 30, 2017

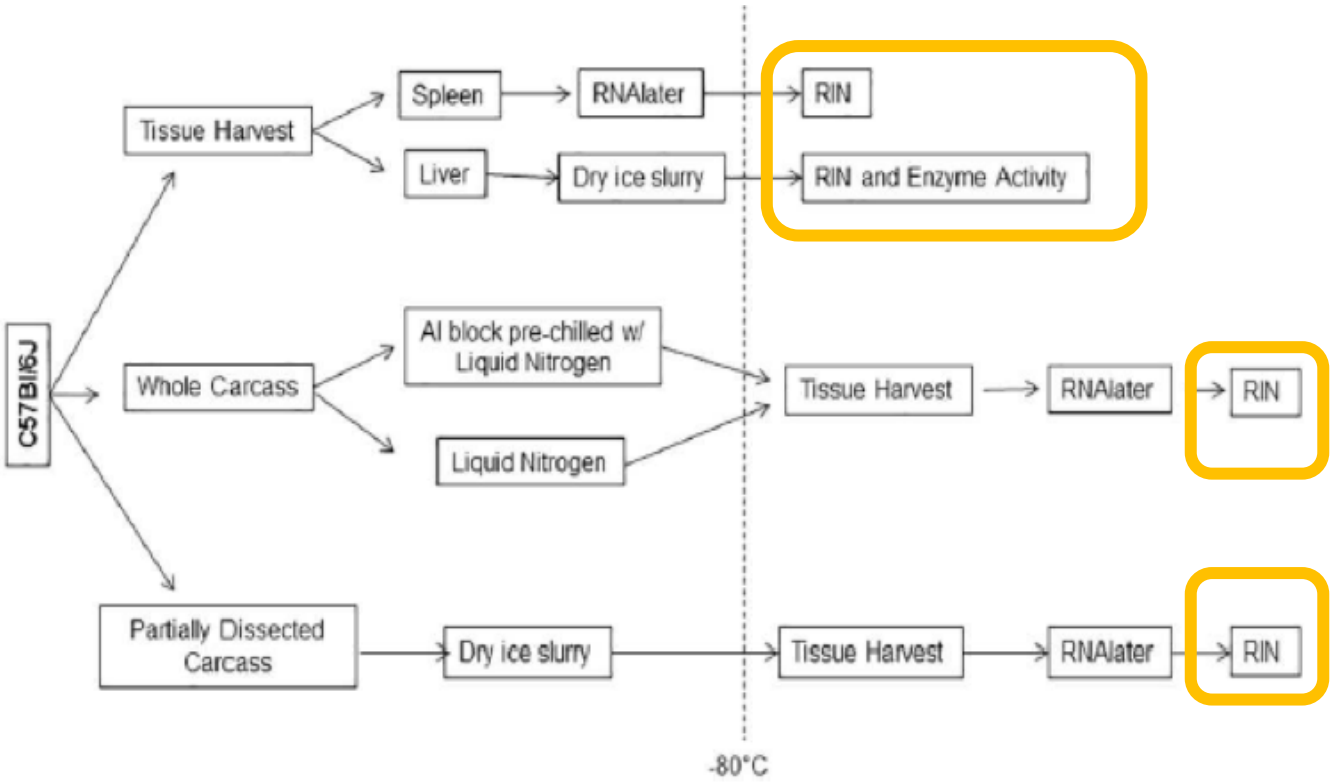
Outline:

- Pre-flight groundbased tests:
  - For gene expression (RIN values)
  - For protein (enzyme activity assays)
- Protocols/methods of preservation to date: freezing profiles from cold stowage
- Overview results from RR1- validation
  - enzyme activities
  - RIN values
- Summary

Pre-flight ground-based testing: Choi et al. PlosOne 2016

Objectives

- Designed to test potential delays in on-orbit dissections and preservation:
  - ➢Select tissues dissected and preserved and frozen using the RR1 protocol
  - ➢intact carcasses frozen at various points post euthanasia
  - ➢more than 20 different tissue types were dissected and analyzed from frozen carcasses



Limitations- proof of principle approach

Limitations: Highly targeted; not 'omic'

-Proteins: Select enzyme activity

-RNA: RIN values only indicate degradation of recovered RNA

RR1 Experimental Design for sample retrieval for validation objective

Primary objectives RR1 Validation Mission related to sample recovery :

Liver	on orbit dissection followed by fast freezing: enzyme activities
Spleen	on orbit dissection and preservation in RNAlater: RNA purification

37 days in µG

NASA VALIDATION MICE
10 C57BL/6J
(female, 16wk old)

→ 2 mice → **Dissected on orbit:**
Liver
Spleen

→ 8 mice → Freeze carcass for BW & sample retrieval **(**post-flight) → **Dissected from frozen carcass:**
Liver
Spleen

21-22 days in µG

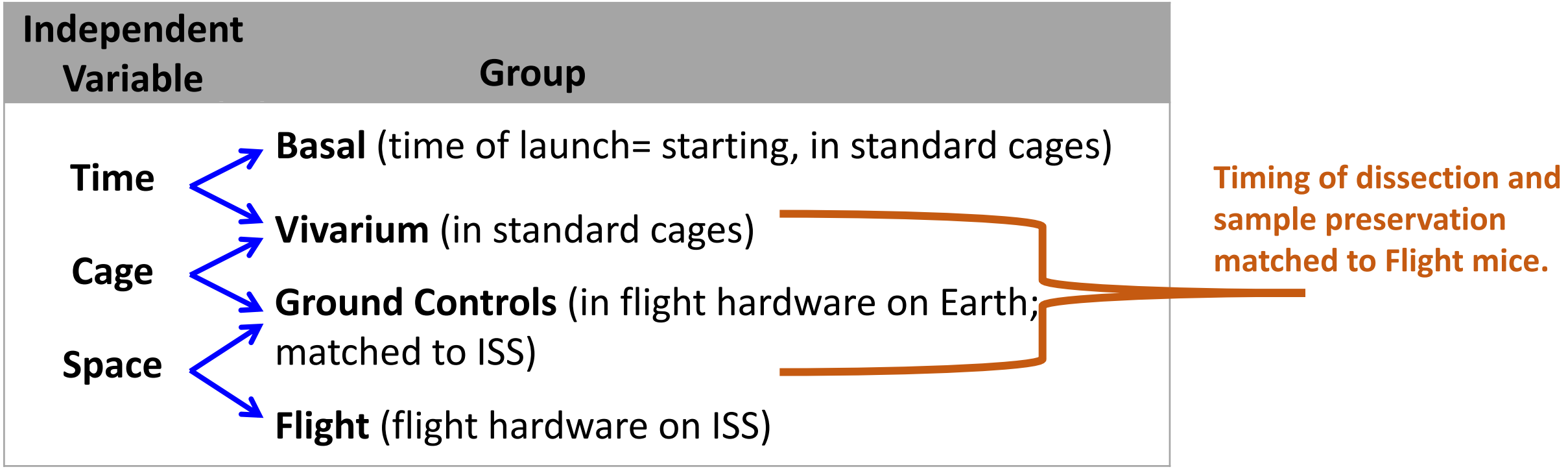
CASIS EXPERIMENTAL MICE
-5 C57Bl/6Tac (Wildtype)
-5 MuRF1 KO
(female, 32 wk old)

→ Hind limb → 10 legs Formalin-fixed

→ **5 WT mice** → **Dissected on orbit for Validation:**
Liver
Spleen

RR1: Validation groups of mice

- 4 separate groups to better understand observed responses to this unique habitat and environment.

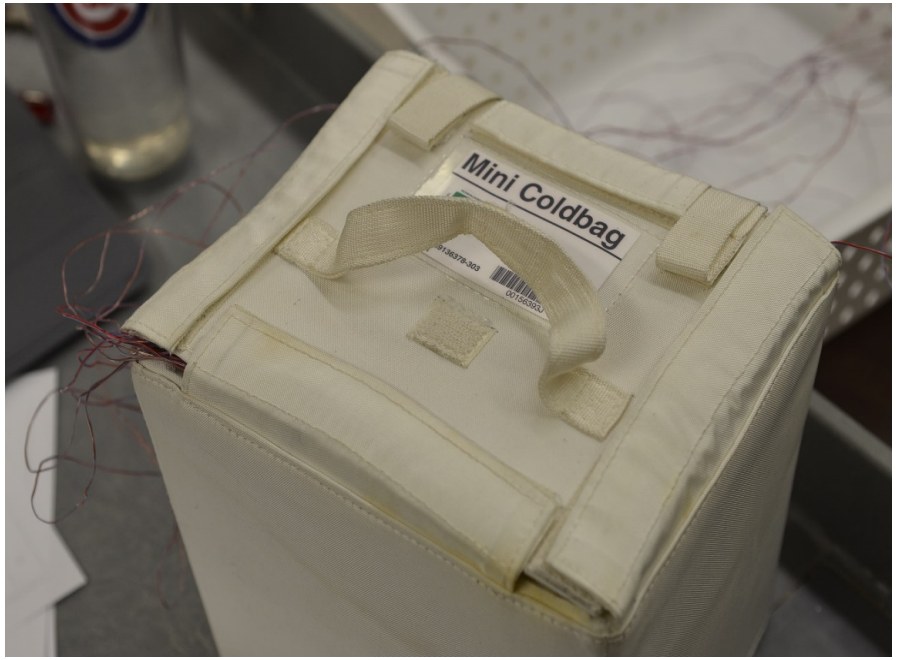


Tissue recovery and analyses: positive controls (frozen in LN2)

RR1: Cold Block and 3 Ice Bricks in MCB (livers and carcasses)

Mini Coldbag (MCB)

- Passive low temperature science storage.



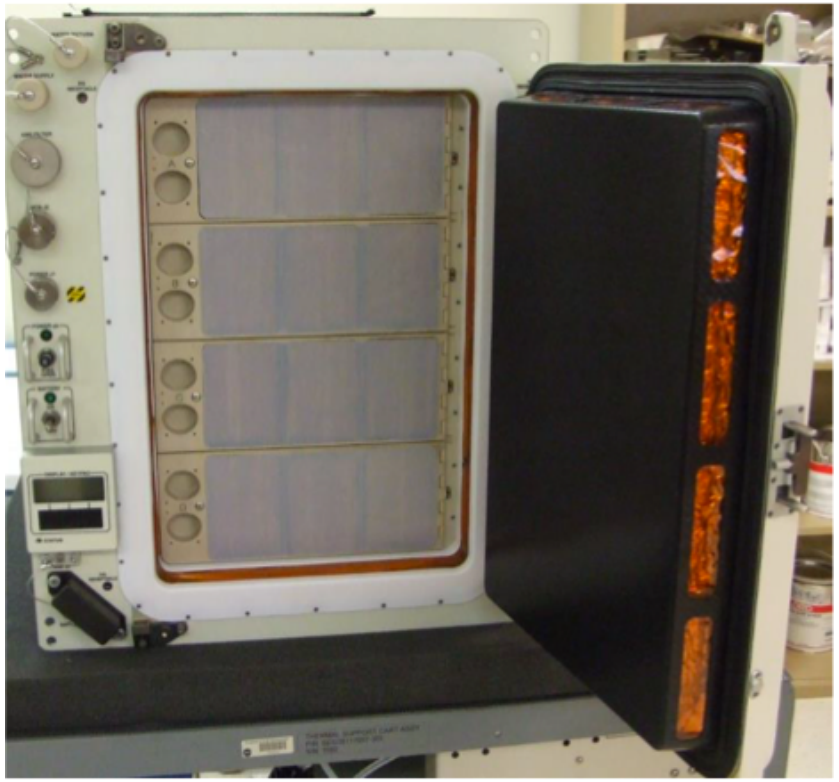
Ice Bricks

- Solid-liquid phase change material in a hard plastic rectangular container compatible with the cold stowage systems. Ice bricks were pre-chilled in the glacier (-130˚C)
- 3 ice bricks were used for each MCB.

Ice Brick assembly

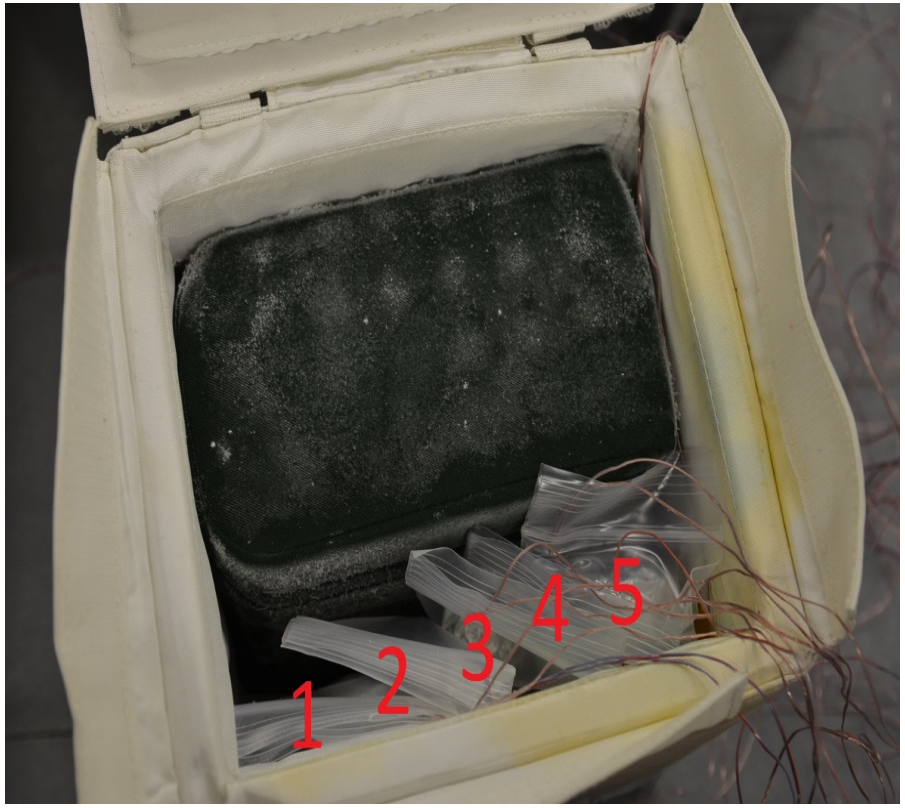
Conditioning Ice Bricks (-130˚C)

Glacier (Flight)



-150˚C freezer at KSC (Ground control)

RR1 Carcass Freezing



- Carcasses were wrapped with two layers of aluminum foil and placed in a ziploc bag
- Placed in MCB containing 3 ice bricks
- 5 Carcasses were placed in metal box and transferred to MELFI
- Notes: ice bricks were swapped out midday

RR1: Livers and Spleens were dissected on orbit

Cold blocks to freeze the livers
(prechilled in Glacier -130°C)

Spleens preserved in RNAlater
(4˚C for 24+ hours then stored in Melfi -95°C)

RR2+: Samples frozen by direct MELFI (-95˚C) insertion

ISS022E011876

Carcass Freezing Rates(MCB vs. MELFI)

MCB with 3 ice bricks

Sample	Mass	Time to Reach -20°C
Day 1 Sample 1	22.0g	31 min
Day 1 Sample 2	22.3g	37 min
Day 1 Sample 3	21.9g	52 min
Day 1 Sample 4	23.6g	71 min
Day 1 Sample 5	22.5g	83 min
Day 2 Sample 1	26.1	36.5 min
Day 2 Sample 2	21.9	32.5 min
Day 2 Sample 3	21.4	43 min
Day 2 Sample 4	23.2	57 min
Day 2 Sample 5	22.4	64.5 min

Direct MELFI insertion

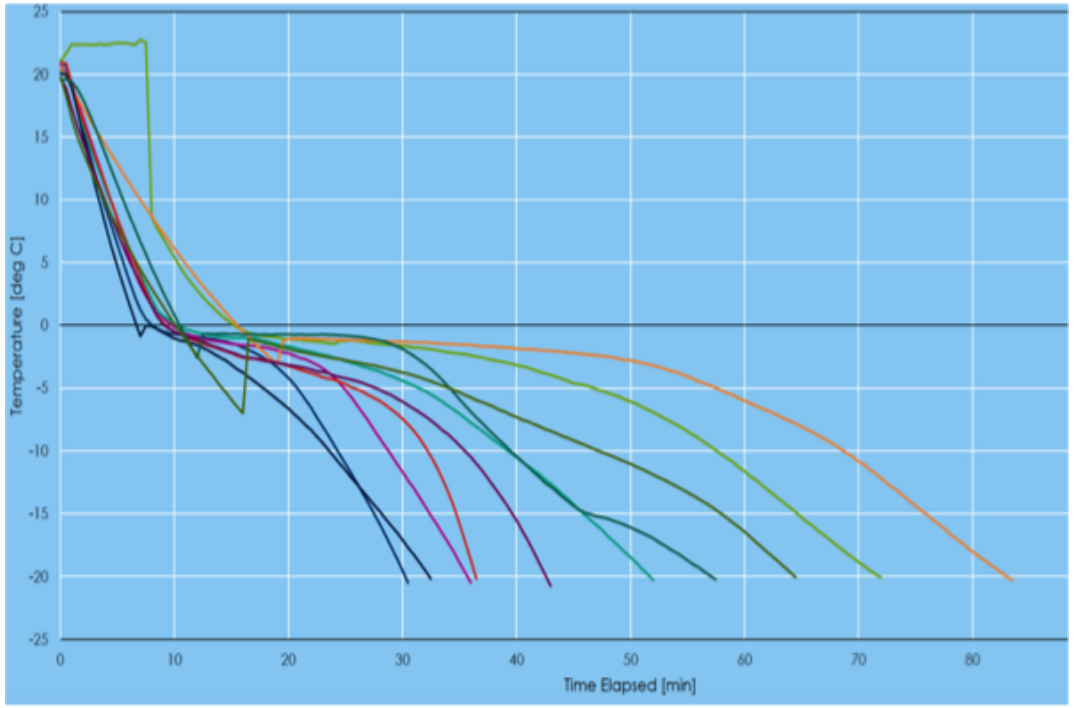
Sample	Mass (g)	Time to Reach -20°C (min)	Time to Reach -80°C (min)
Sample 1	33.6	38	101.5
Sample 2	28.14	38	106.5
Sample 3	26.97	41	87.5
Sample 4	25.33	37	175.5
Sample 5	29.9	40	142
Sample 6	30.61	43.5	108
Sample 7	26.5	39.5	88.5
Sample 8	24.1	37.5	77

Mouse carcass freezing times per RR1 on orbit protocol

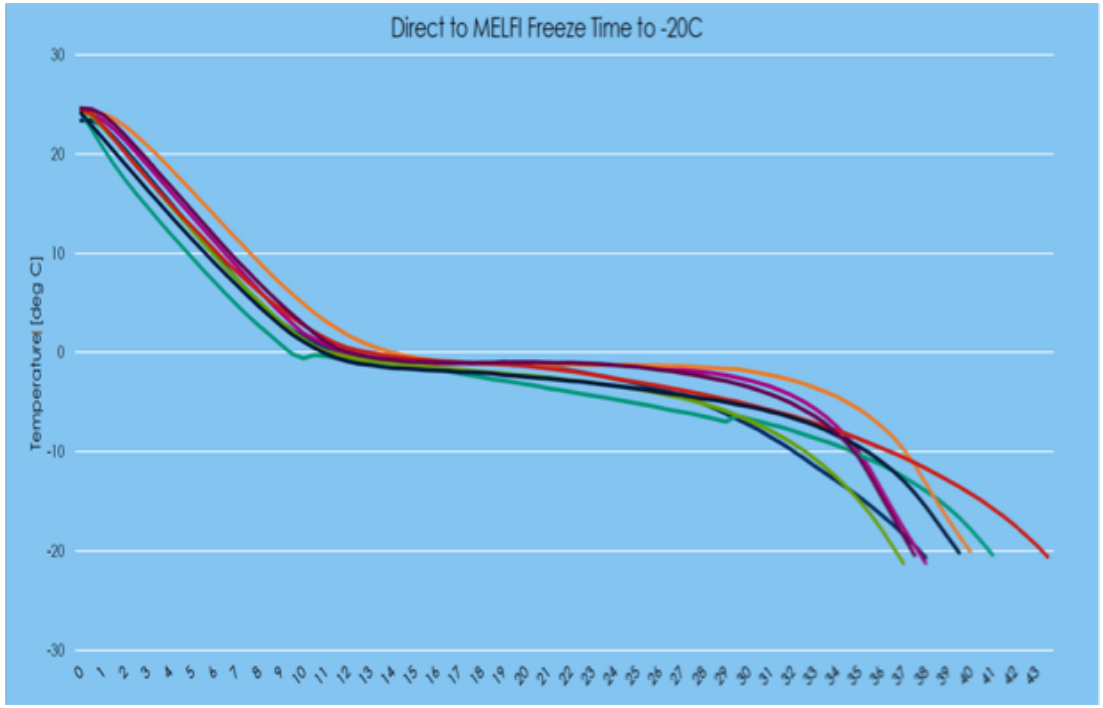
Data provided by JSC Cold Stowage

Sample Freezing time to -20˚C (~25g Carcasses): MCB vs. MELFI

MCB with 3 Ice Bricks



Direct MELFI insert



Data provided by JSC Cold Stowage

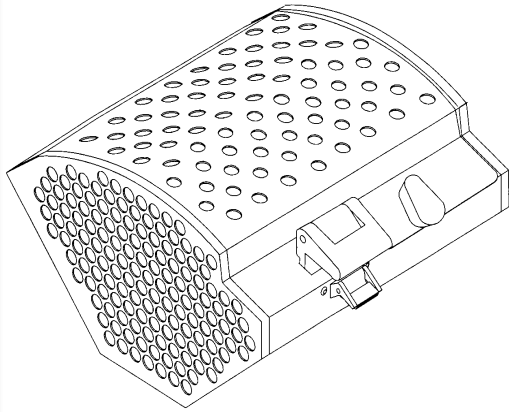
Tissue freezing rates - Cold block and 3 ice bricks in MCB

Sample	Mass (g)*	Time to Reach -20°C (sec)	Time to Reach -80°C (sec)
Sample 1-1	2.91	100	225
Sample 1-2	3.05	110	180
Sample 1-3	2.97	150	290
Sample 1-4	3.14	130	285
Sample 1-5	2.88	145	395
Sample 1-6	1.88	150	255
Sample 1-7	1.84	150	290
Sample 1-8	1.72	140	400
Sample 2-1	3.07	105	240
Sample 2-2	3.04	120	210
Sample 2-3	3.11	130	260
Sample 2-4	3.1	145	580
Sample 2-5	3.04	140	410
Sample 2-6	1.81	150	280
Sample 2-7	1.98	155	315
Sample 2-8	1.6	170	640

Data provided by JSC Cold Stowage

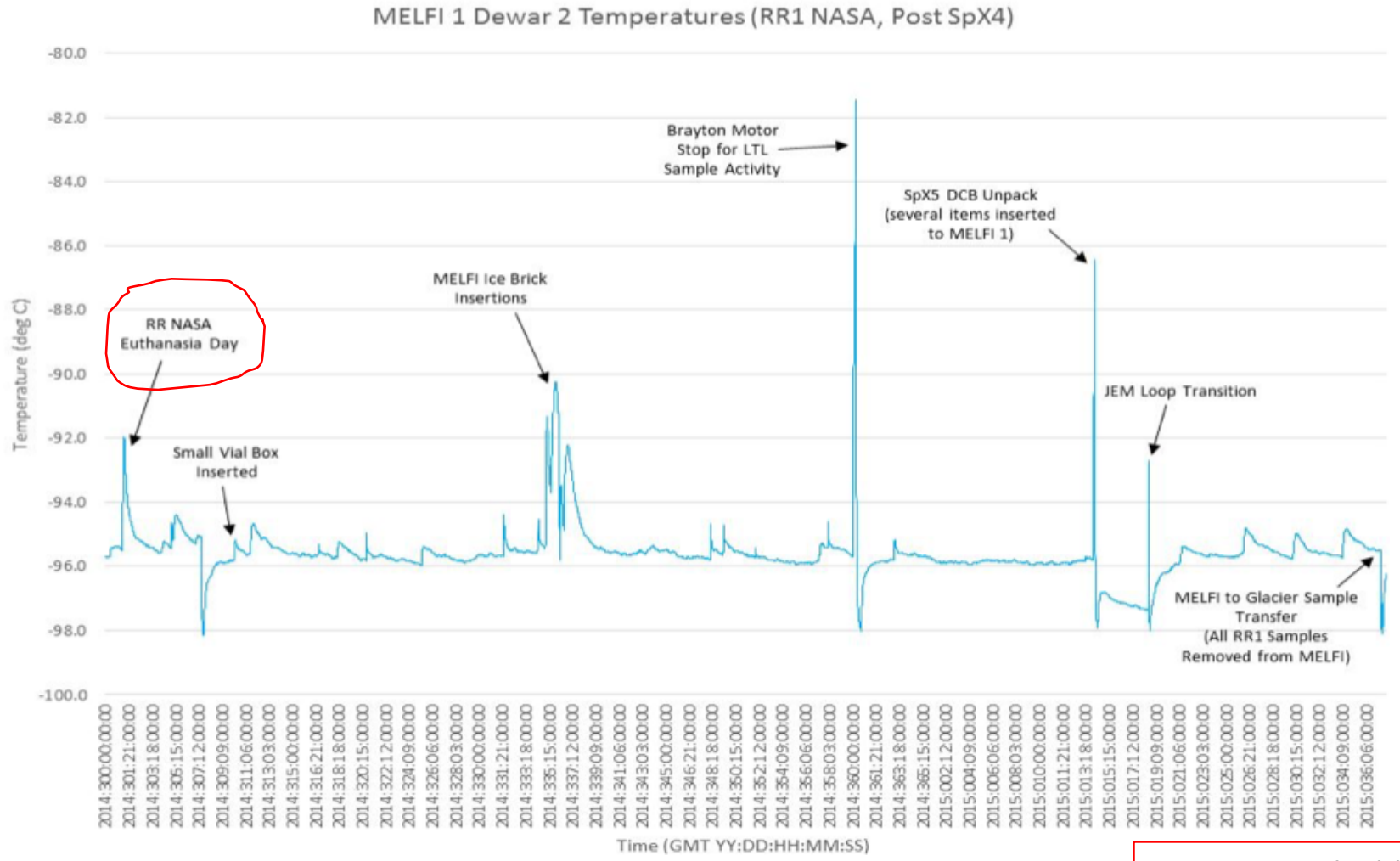
Samples stored in MELFI until return to Earth

On orbit: MELFI (-95˚C)



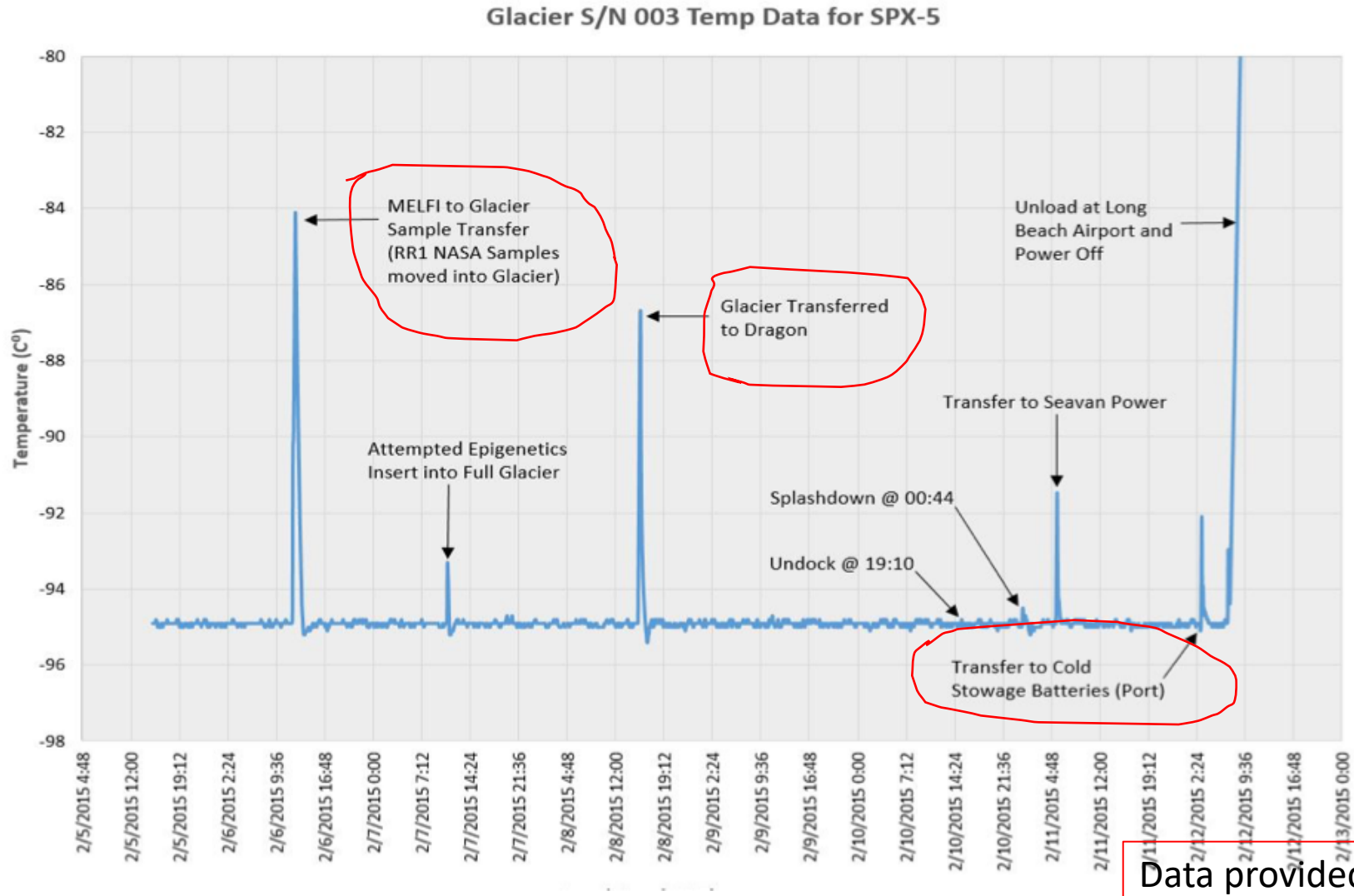
Ground: -80˚C

RR1:Temperature profile between MELFI insertion of samples and MELFI to Glacier transfer for return



Data provided by JSC Cold Stowage

RR1:Temperature profile during return



Data provided by JSC Cold Stowage

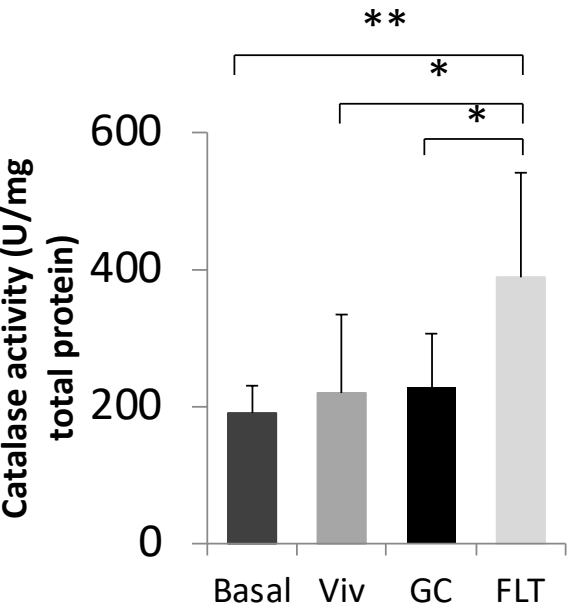
RR1 Temperature profile during shipping to Ames

Data collected by Ames RRP

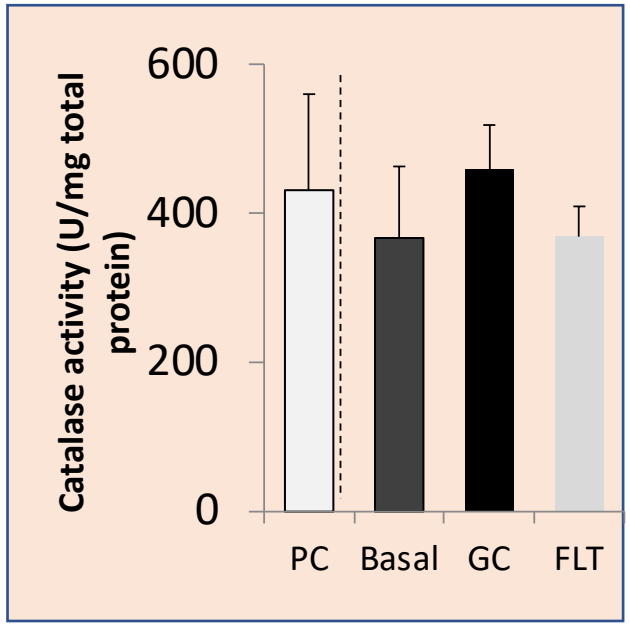
RR1 Validation results

RR1 Liver enzyme results from Validation and Experimental Mice

Validation (NASA)
Frozen Carcass



Experimental (CASIS)
On Orbit dissected



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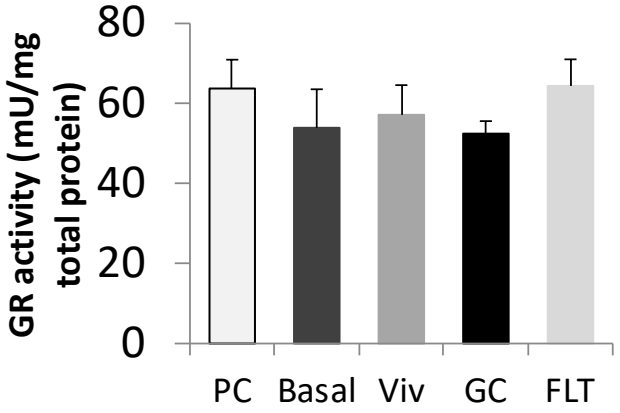
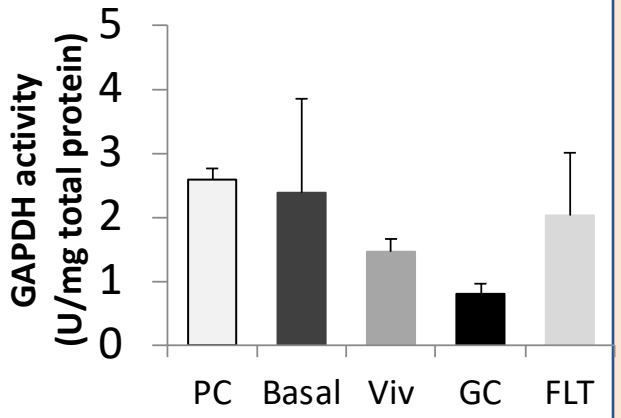
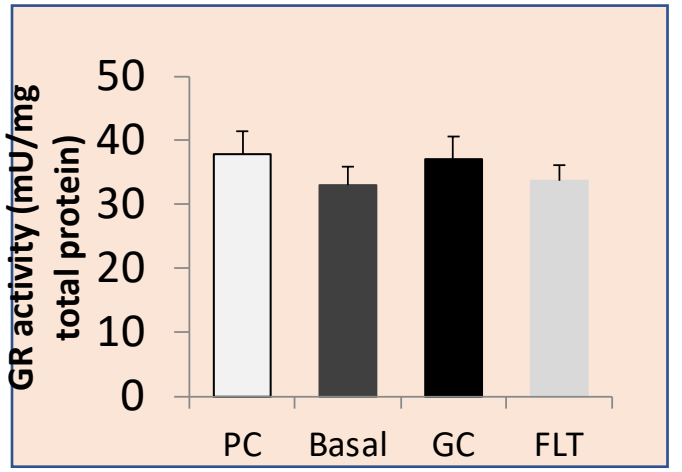
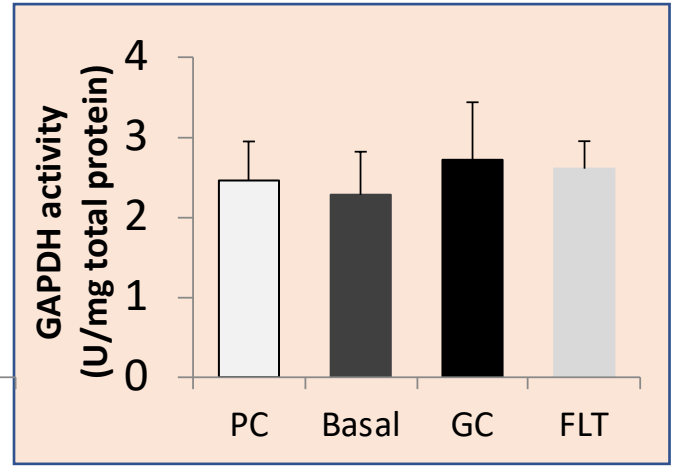




Experimental (CASIS)
On Orbit dissected





RNA quality

Liver RNA RIN

	On-Orbit Dissected Validation Mice		On-Orbit Dissected Experimental Mice		Validation Frozen Carcasses	
	Sample size (n)	RIN (mean ± SD)*	Sample size (n)	RIN (mean ± SD)	Sample size (n)	RIN (mean ± SD)
Positive Control	3	9.67 ± 0.31	5	8.56 ± 0.59	5	9.30 ± 0.17
Basal	2	9.10	5	8.66 ± 0.13	7	8.59 ± 0.62
Vivarium	2	8.85	NA	NA	6	8.24 ± 0.57
Ground Controls	2	8.45	5	8.62 ± 0.77	6	8.19 ± 0.72
Flight	2	9.15	5	8.72 ± 0.50	6	8.41 ± 0.95

Spleen RNA RIN

	On-Orbit Dissected Validation Mice		On-Orbit Dissected Experimental Mice	
	Sample size (n)	RIN (mean ± SD)*	Sample size (n)	RIN (mean ± SD)
Positive Control	3	9.90 ± 0.10	5	9.82 ± 0.17
Basal	2	9.80 ± 0.14	5	9.62 ± 0.40
Vivarium	2	9.10 ± 1.27	NA	NA
Ground Controls	2	9.55	5	9.28 ± 0.55
Flight	2	9.10	5	9.31 ± 0.45

Summary and Conclusion

- Select tissues can be utilized for gene expression, protein, histology, immunohistochemistry assays, despite being retrieved from frozen carcasses
  - Gene expression: despite high RIN values, evidence from RNAseq analysis for artifact from preservation method

- These results expand potential science return from valuable and limited rodent experiments in space