

**Spaceflight increases sarcoplasmic reticulum Ca²⁺ leak and this cannot be counteracted with BuOE
treatment**

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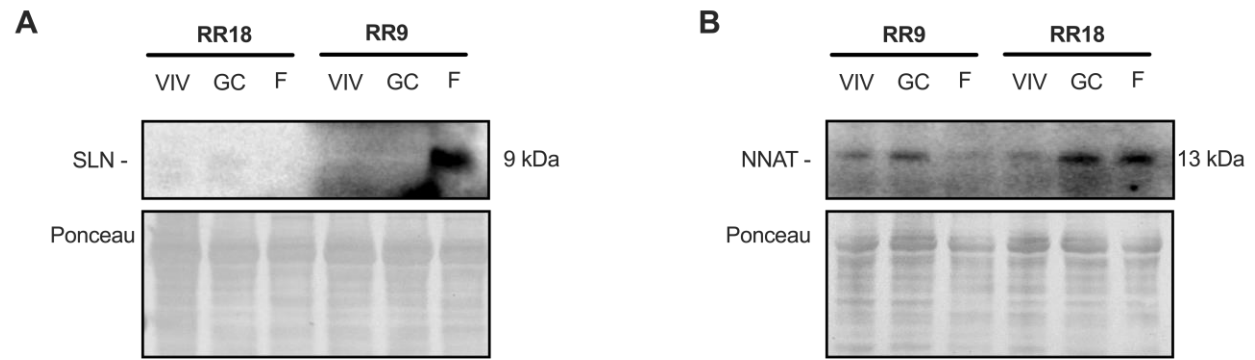
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SUPPLEMENTARY MATERIAL

Supplemental Table 1. Protein specific Western blotting protocols and materials.

Target	Protein Loaded (µg)	Type of Gel	Membrane	Primary Antibody Dilution	Primary Antibody Details
SERCA1a	10	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:5000	MA3-912, ThermoFisher Scientific
SERCA2a	2.5	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:5000	MA3-919, ThermoFisher Scientific
RYR	10	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:2000	MA3-925, ThermoFisher Scientific
p-RYR	10	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:2000	AF3703, Affinity Biotech
4-HNE	10	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:5000	AB5605, Millipore Sigma
SOD	10	BioRad PreCast TGX 4-15% gradient gel	PVDF	1:5000	NB100- 1992SS, Novus
SLN	25	Homemade tricine	Nitrocellulose	1:250	ABT13, Sigma Aldrich
NNAT	15	Homemade tricine	PVDF	1:1000	78122S, Cell Signaling Technology
PLN	10	Homemade tricine	PVDF	1:2000	MA3-922, ThermoFisher Scientific

Abbreviations: sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA); ryanodine receptor (RYR); 4-hydroxynonenal (4-HNE); superoxide dismutase (SOD); sarcoplipin (SLN); neuronatin (NNAT); phospholamban (PLN); polyvinylidene fluoride (PVDF)



Supplemental Figure 1. Representative Western blots of SLN and NNAT in RR-9 vs RR-18 soleus.

Representative images show differential responses to spaceflight between the RR-9 and RR-18 missions in SLN (**A**) and NNAT (**B**).