

Supplementary Materials

McMahon, *et al.*, "Savory Signaling: T1R umami receptor modulates endoplasmic reticulum calcium store content and release dynamics in airway epithelial cells."

Antibodies Used	Source	Catalogue Number
anti-T1R1	ThermoFisher Scientific	PA528771
anti-T1R3	Abcam	ab150525
Cell Lines Used	Source	Catalogue Number
Beas-2B	ATCC	CRL-9609
Primary human bronchial epithelial cells	Lonza	CC-2450
Primary nasal epithelial cells	This Study	N/A
Chemicals Used	Source	Catalogue Number
Bovine Serum Albumin	Millipore Sigma	A2153
CellEvent Caspase 3/7 Reagent	ThermoFisher Scientific	C10423
DAF-FM diacetate	ThermoFisher Scientific	D23844
Denatonium Benzoate	Millipore Sigma	D5765
Fluo-8 AM	Abcam	ab142773
Forskolin	Millipore Sigma	F3917
Fura-2 AM	ThermoFisher Scientific	F1221
Isoproterenol	Millipore Sigma	I6504
Lipofectamine 3000	ThermoFisher Scientific	L3000075
MEM Amino Acids	ThermoFisher Scientific	11130051
Non-Essential Amino Acids	ThermoFisher Scientific	11140050
Thapsigargin	Cayman Chemical	10522
UTP	ThermoFisher Scientific	AAJ23160-03
Recombinant DNA - Function	Source	Catalogue Number
AKAR4 - intracellular PKA activity	Addgene	61619
AKAR4-nls - nuclear PKA activity	Addgene	138217
Flamindo2 - intracellular cAMP	Addgene	73938
nls-Flamindo2 - nuclear cAMP	Addgene	73939
RNAi used	Source	Catalogue Number
TAS1R1 DsiRNA Kit	Integrated DNA Technologies	hs.Ri.TAS1R1.13
TAS1R3 DsiRNA Kit	Integrated DNA Technologies	hs.Ri.TAS1R3.13
Taqman Probes for qPCR	Source	Catalogue Number
TAS1R1	ThermoFisher Scientific	Hs01547926_g1
TAS1R2	ThermoFisher Scientific	Hs00541095_m1
TAS1R3	ThermoFisher Scientific	Hs00877446_g1
UBC	ThermoFisher Scientific	Hs01871556_s1

Table S1. A list of reagents used in this study.

a**MEM Amino Acids, Essential Amino Acids**

ThermoFisher Product Number 11130051

Amino Acid	1x Concentration (mM)
L-Arginine hydrochloride	0.60
L-Cystine	0.10
L-Histidine hydrochloride-H ₂ O	0.20
L-Isoleucine	0.40
L-Leucine	0.40
L-Lysine hydrochloride	0.40
L-Methionine	0.10
L-Phenylalanine	0.20
L-Threonine	0.40
L-Tryptophan	0.05
L-Tyrosine	0.20
L-Valine	0.40

b**MEM Non-Essential Amino Acids**

ThermoFisher Product Number 11140050

Amino Acid	1x Concentration (mM)
Glycine	0.1
L-Alanine	0.1
L-Asparagine	0.1
L-Aspartic acid	0.1
L-Glutamic Acid	0.1
L-Proline	0.1
L-Serine	0.1

Table S2. Formulations of 1x MEM AA (a) and 1x NEAA (b) used in this study.

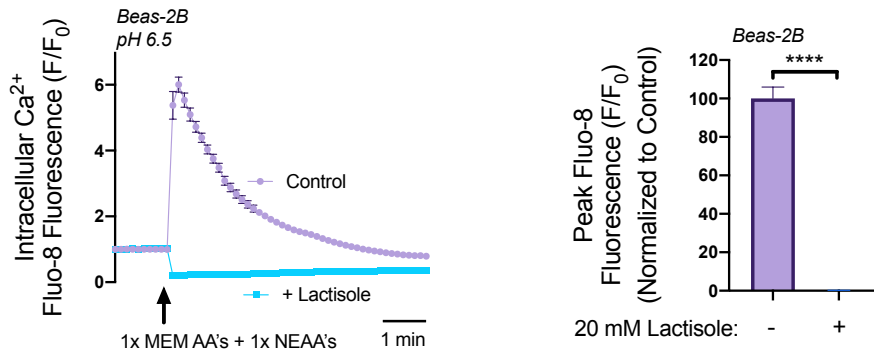
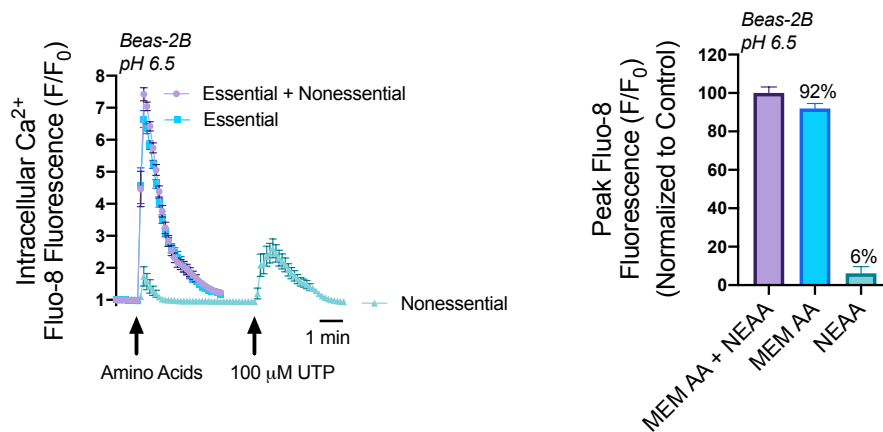
a**b**

Figure S1. Amino acids induce Ca²⁺ elevations in Beas-2B's at pH 6.5. (a) Beas-2B's were loaded with Ca²⁺ detecting dye Fluo-8 AM and pre-treated with 20 mM lactisole for 1 hour then stimulated with a mixture of 1x MEM AA and 1x NEAA's. At pH 6.5 1x MEM AA's and 1x NEAA stimulate a Ca²⁺ response that is inhibited by lactisole. (b) Maintaining a pH of 6.5, the majority of the Ca²⁺ elevations (approximately 92%) are due to components of the 1x MEM AA mixture while 1x NEAA contributed minimally to Ca²⁺ signaling pathways (approximately 6% of the combined release). Significance determined by t-test *****p* < 0.0001

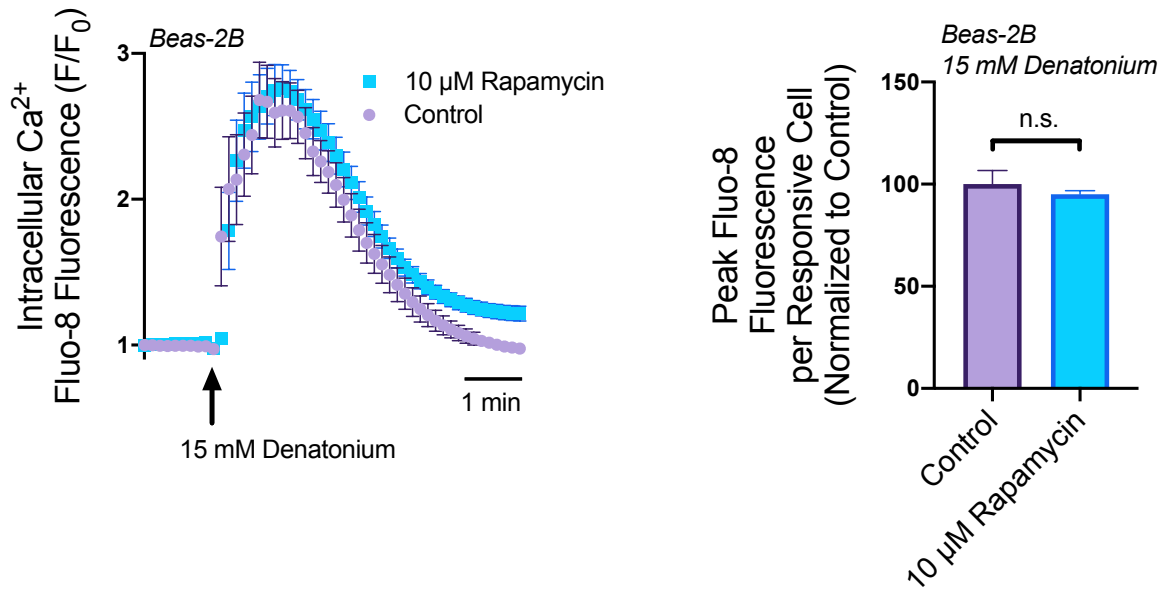


Figure S2. mTORC1 pathways do not impact ER Ca^{2+} content. Beas-2B's loaded with Fluo-8 AM were treated with 10 μM of rapamycin for 1 hour then stimulated with 15 mM denatonium. Rapamycin had no impact on the population's peak Ca^{2+} levels or the peak Ca^{2+} release per cell, showing that there was no observable effect on Ca^{2+} signaling pathways. Significance determined by t-test 'n.s.' represents no significance.