Chronic Mild Thermal Stress Stimulates Tumor Growth and Depresses the Anti-Tumor Immune Response

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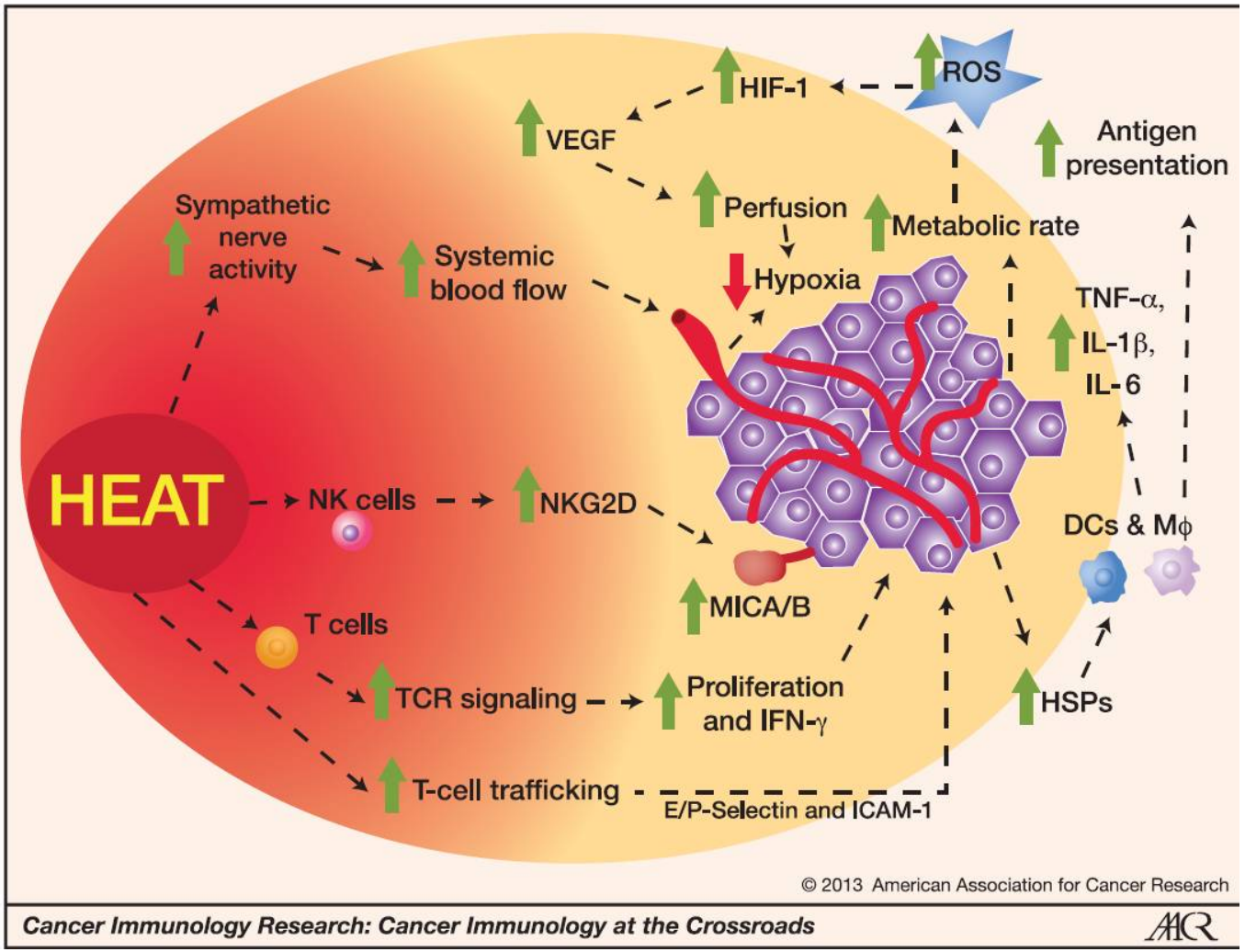
ICNIRP/WHO Workshop
"A Closer look at the thresholds of thermal damage"
Istanbul, Turkey, May 26-28, 2015

How is the immune system regulated by body temperature?

Mild (Fever-Range) Hyperthermia Affects Many Aspects of the Tumor Microenvironment



Repasky, Evans, Dewhirst,
Cancer Immunology Research, 2013

THERMOREGULATION- A unique automomic system

- Evolved to regulate a stable core over wide range of ambient temperatures, heat loads from work, and fever
- One of the only systems that also relies on behavior and conscious awareness of the environment to achieve regulation

Effectiveness of HumanThermoregulation

The stress response

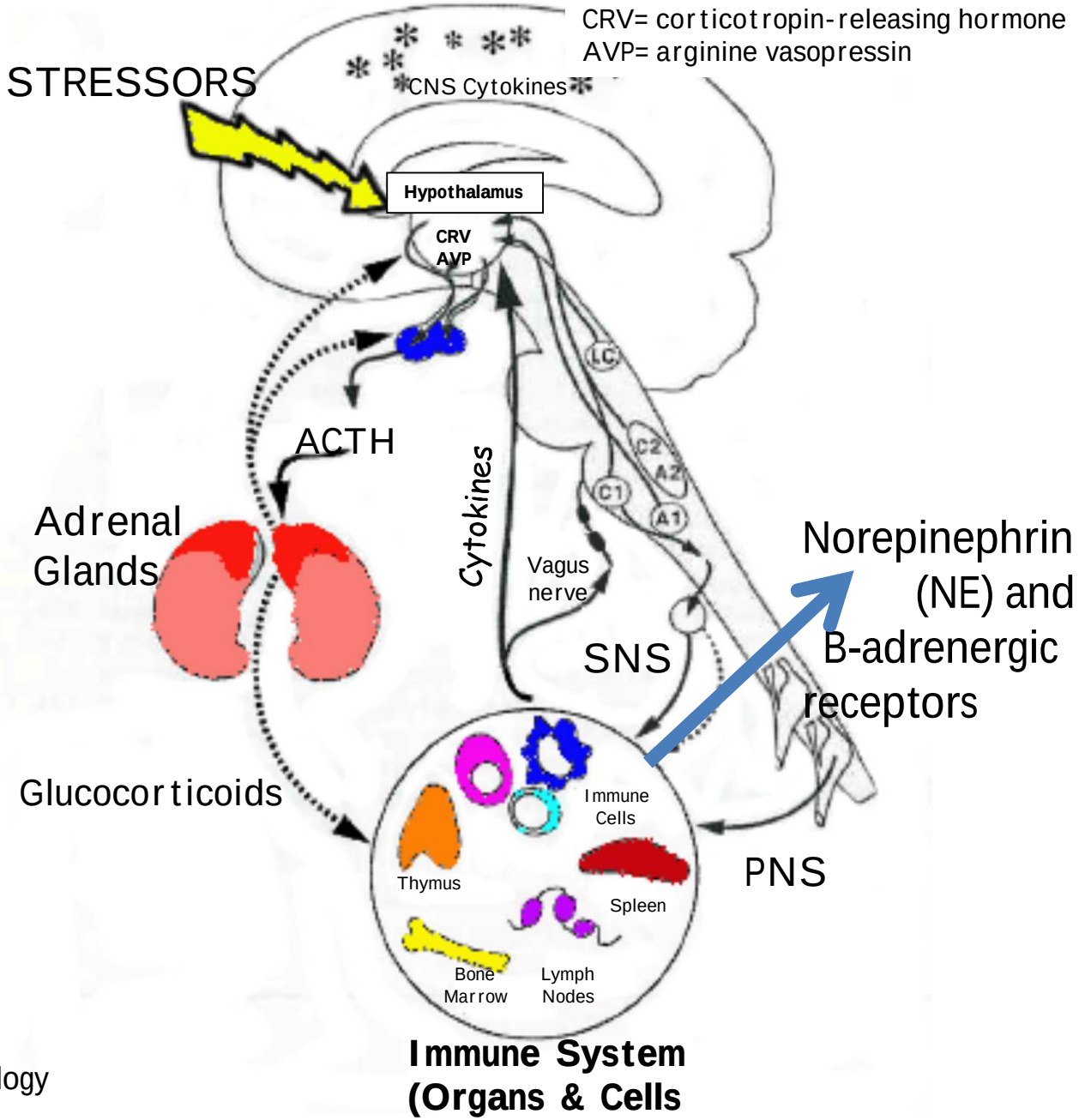
- Integrated definition
  - Stressor (internal or external stimulus)
  - Stress perception in the brain
  - Physiologic adaptions ("fight-or-flight")
- Internal stimuli
  - Emotions (depression, fear)
  - Anxiety (crowding, isolation)
- External stimuli
  - Chemical (toxins)
  - Biological (infection, inflammation, metabolic)
  - **Physical (temperature, pain)**

Sympathetic Nervous System (SNS)-Norepinephrine (adrenalin)

Hypothalamic-Pituitary-Adrenal axis (HPA-axis)

Dhabhar et al, Immunol Res 2014
Joel et al, Nature Reviews Neuroscience 2009
Glaser et al, Nature Reviews Immunology 2005

Hormonal and "hard-wired" mediators of stress

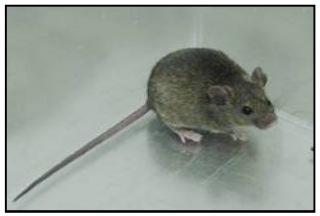


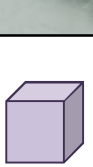
Paul- Fundamental Immunology

Sensitivity to ambient temperature depends on surface/volume ratio-

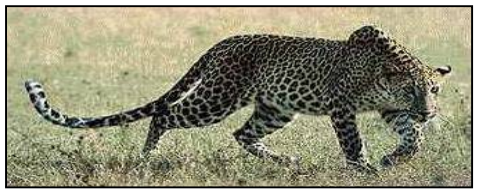
Mice are very sensitive to ambient temperature

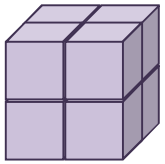
~37 ºC



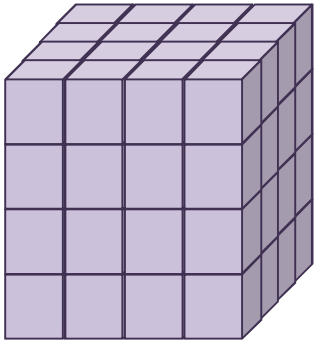


~37 ºC





~37 ºC



Surface Area to Volume Ratio	6	3	1.5

Mice easily gain, *or lose*, heat from their environment

Adapted from Blumberg, *Body Heat: Temperature and Life on Earth*, 2002


HIGH-MPG CARS
Gasoline-Engine Innovations
STOPPING INFECTIONS
New Ways to Thwart Bacterial Invaders
SCIENTIFIC AMERICAN
Mysteries of How a STAR IS BORN
Why Humans Have No Fur
And How Evolving Bare Skin Led to Big Brains
"Impossible" Colors
See Hues That Can't Exist
Plus:
Seafloor Oases
Whale Carcasses Sustain Ecosystems

Housing temperature for laboratory mice—

An unexpected tool for studying cold stress and cancer

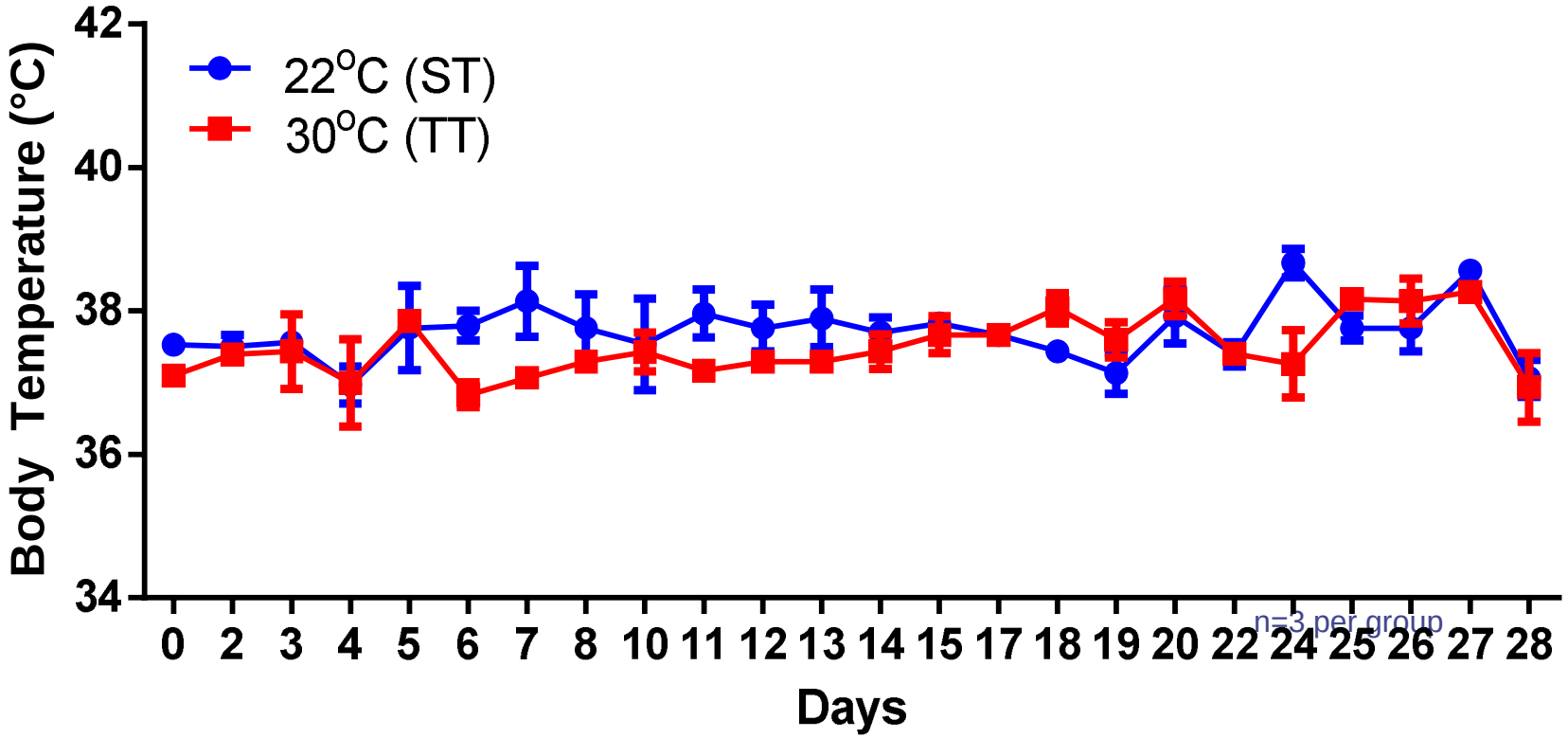
- The thermoneutral temperature for laboratory mice is between 29-31°C (~84-88°F)
  - At thermoneutrality, little or no extra metabolism is required to maintain body temperature.

    (Gordon, *Thermoregulation in Rodents*, 1993; Cannon, *Physiological Revs.*, 2004)
- But IACUC certified facilities must select and maintain a temperature between 20-26°C (~68-78°; until 2011, 18-24 C permitted). (*Guide for the Care and Use of Laboratory Animals,* 8th Ed, 2011)
  - The standard temperature ranges in Europe and Asia are even lower (approx.18-24°C).
  - The temperature in the animal facility at RPCI is maintained between 22-23°C (approx. 72 ^{o}C)

Mice in a huddle- A typical scene in the mouse room at ST

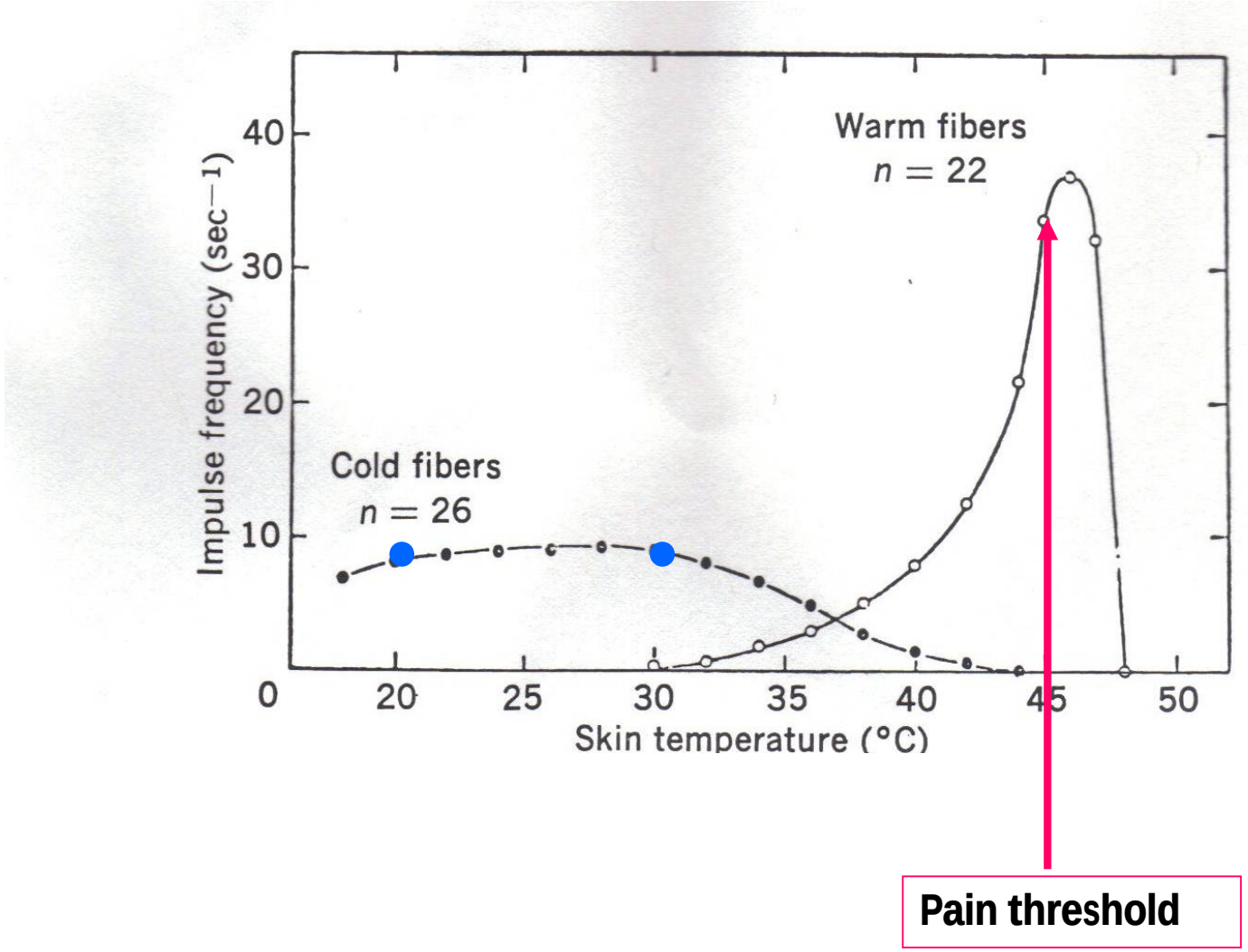
Photo by K. Kokolus and S. Sexton,
RPCI

Mice at ST and TT maintain a normal body temperature



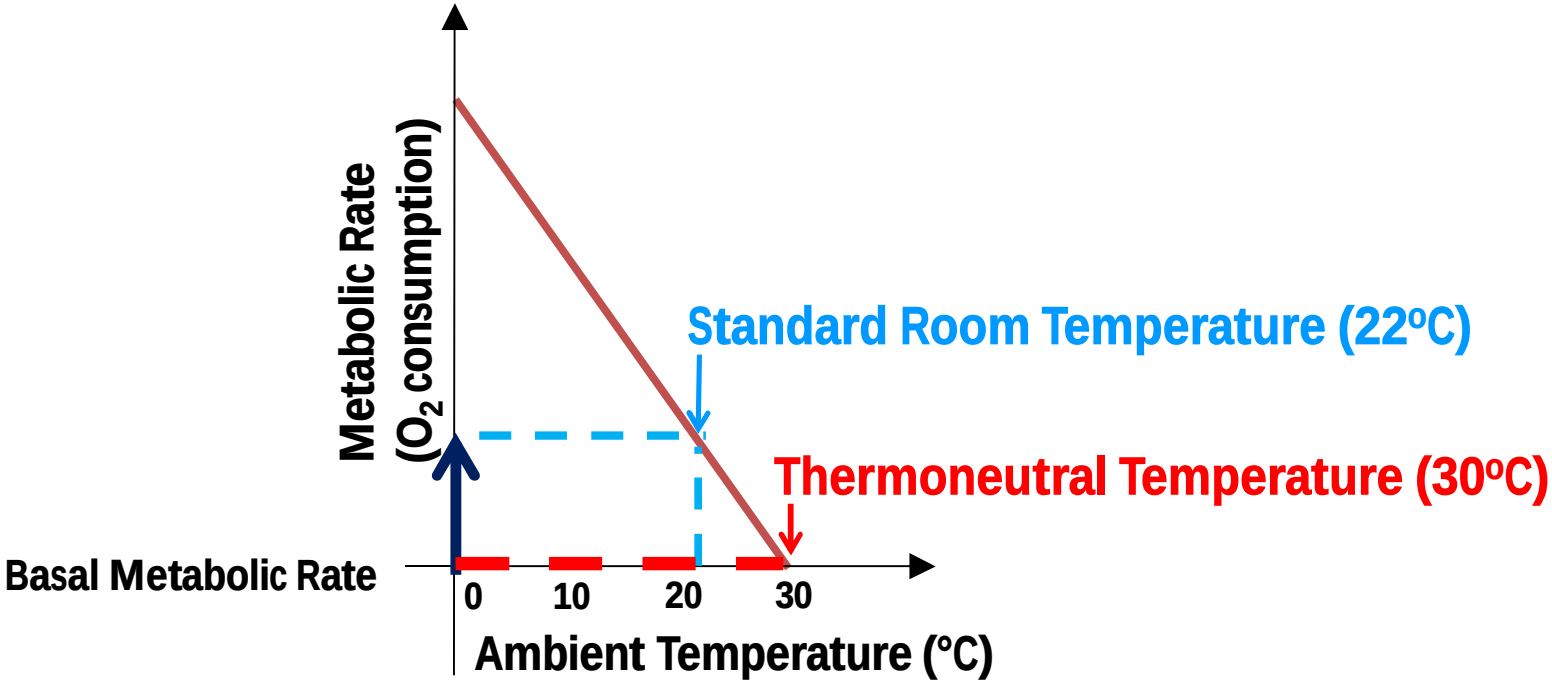
Kokolus et al, PNAS 2013

STATIC DISCHARGE RATE OF CUTANEOUS WARM AND COLD FIBERS



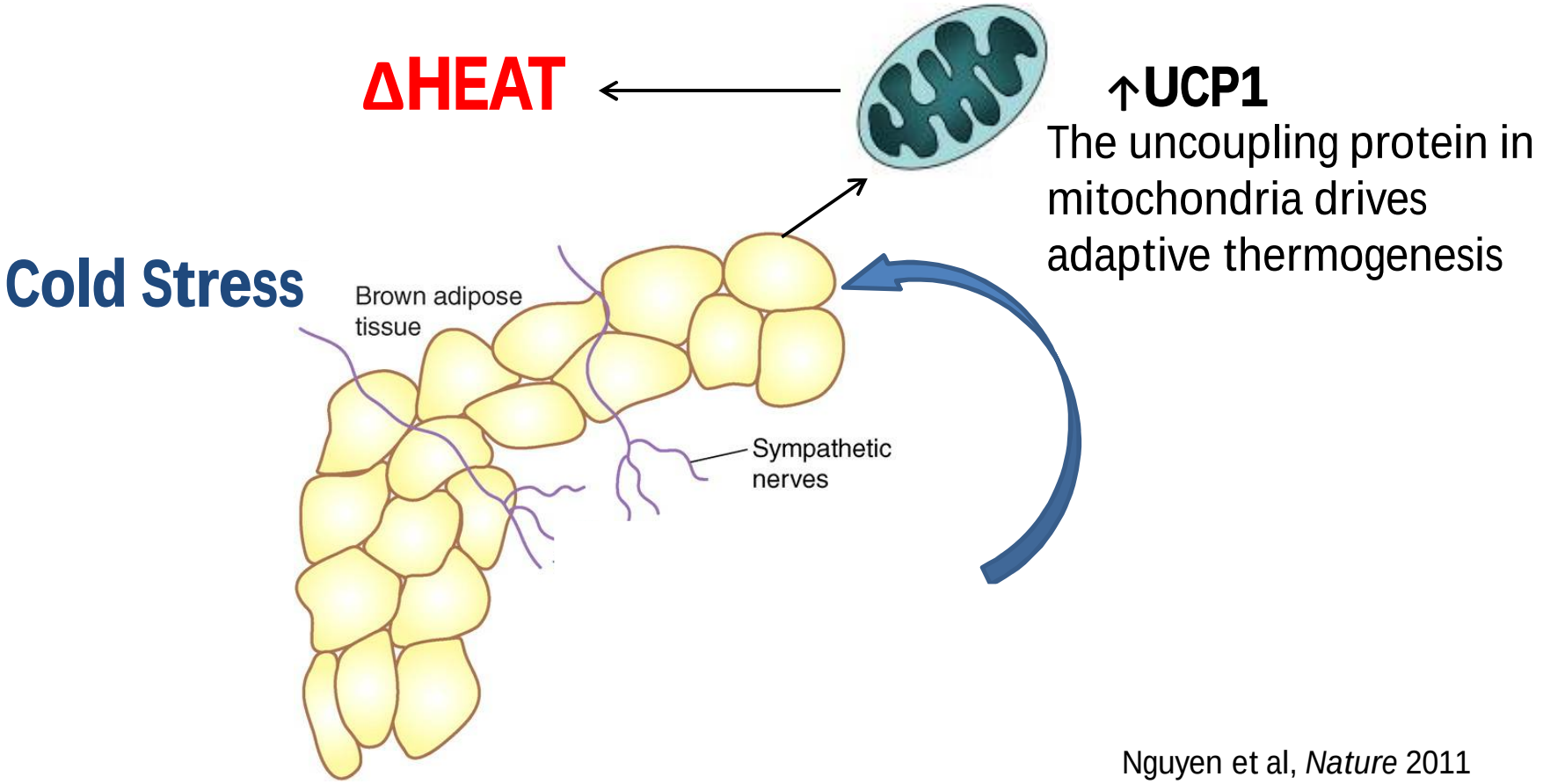
Hensel, 1969

Maintaining a Normal Body Temperature (37°C) in Mice Requires a Significant Increase in Metabolic Rate when Housed ST



Adapted from Specter et al, Handbook of Biological Data 1956
Silva et al, Physiological Reviews 2006
Canon et al, Journal of Experimental Biology 2011

Cold Stress Induces Norepinephrine Production to Facilitate Heat Production

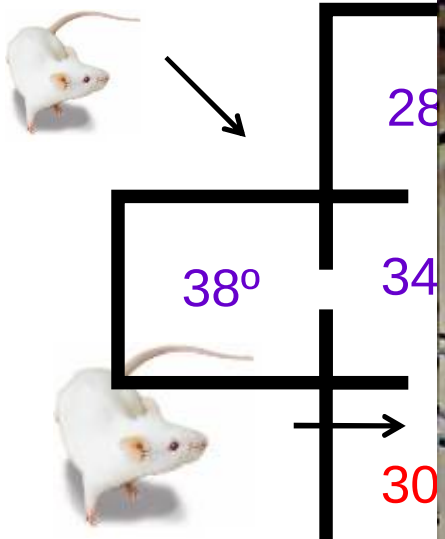


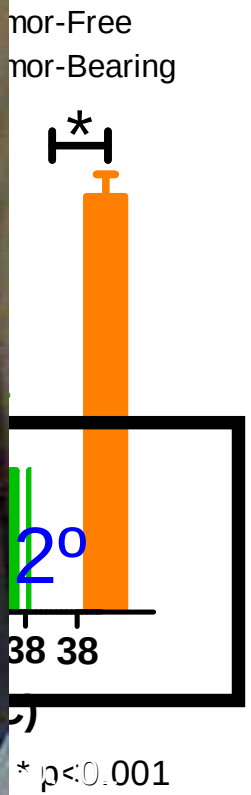
Nguyen et al, *Nature* 2011

Cannon et al, Physiology Reviews 2006
Adapted from Karp et al, Journal of Experimental Medicine 2012

Laboratory mice feel even more *cold* after tumor implantation!

Using a thermal preference apparatus to assess thermal comfort

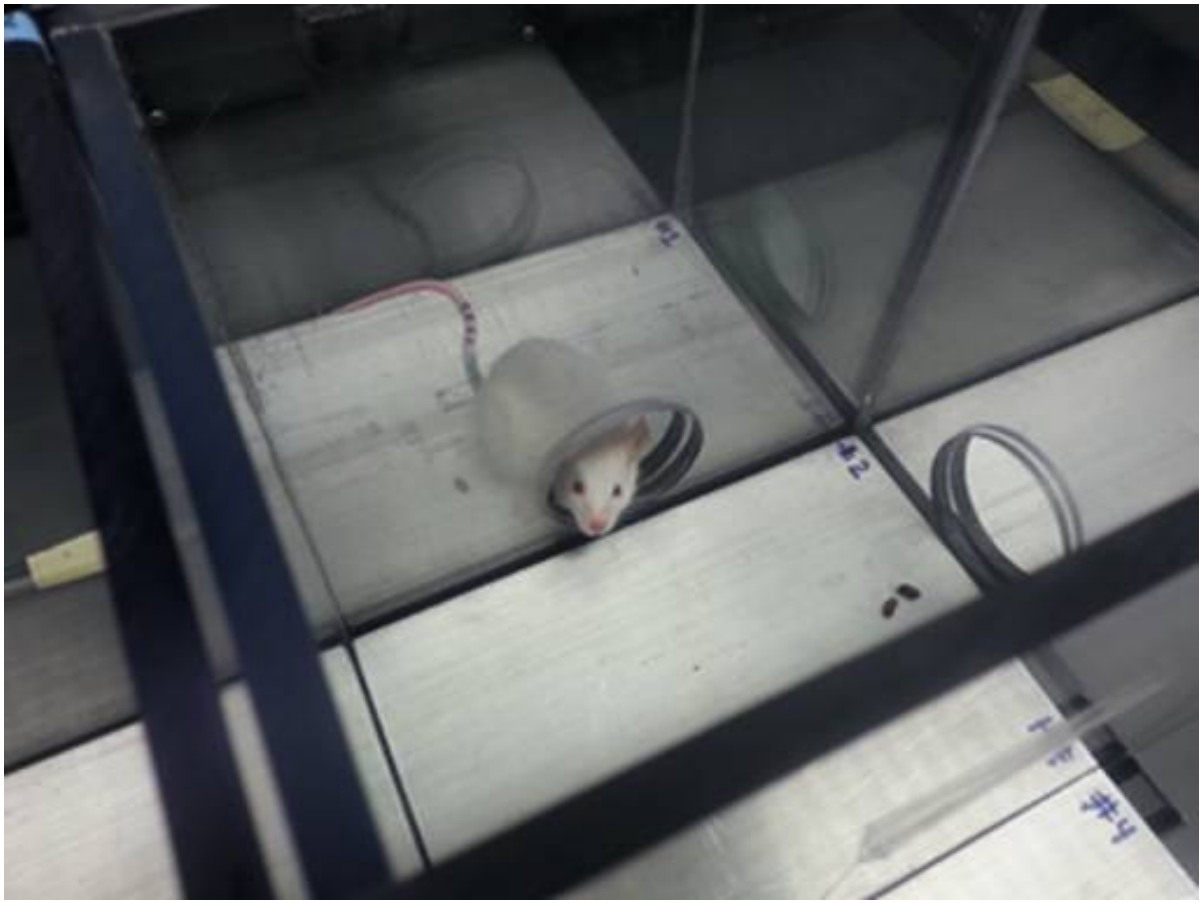




Katie Kokolus, PhD

n=5, p=0.01

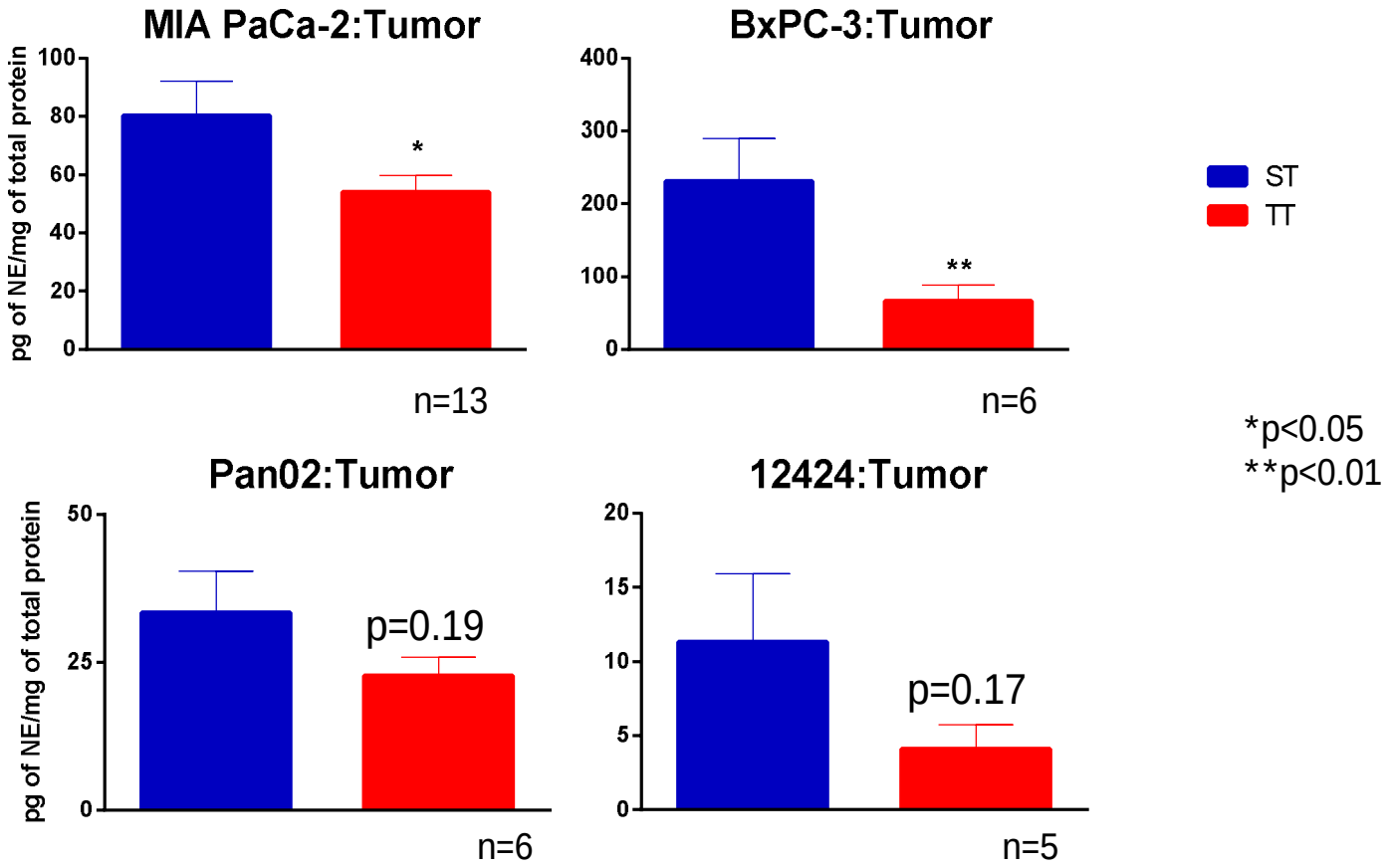
Older mice (> 18 months) prefer a warmer temperature (~34 ºC)



Bonnie Hylander, PhD

Plasma and Tumor Norepinephrine Levels are Increased in Mice Housed at ST compared with TT

Naïve and tumor-bearing mice maintained at ST and TT for 3-4 weeks → Collect plasma and tumor lysates → Analyze by ELISA for norepinephrine concentrations

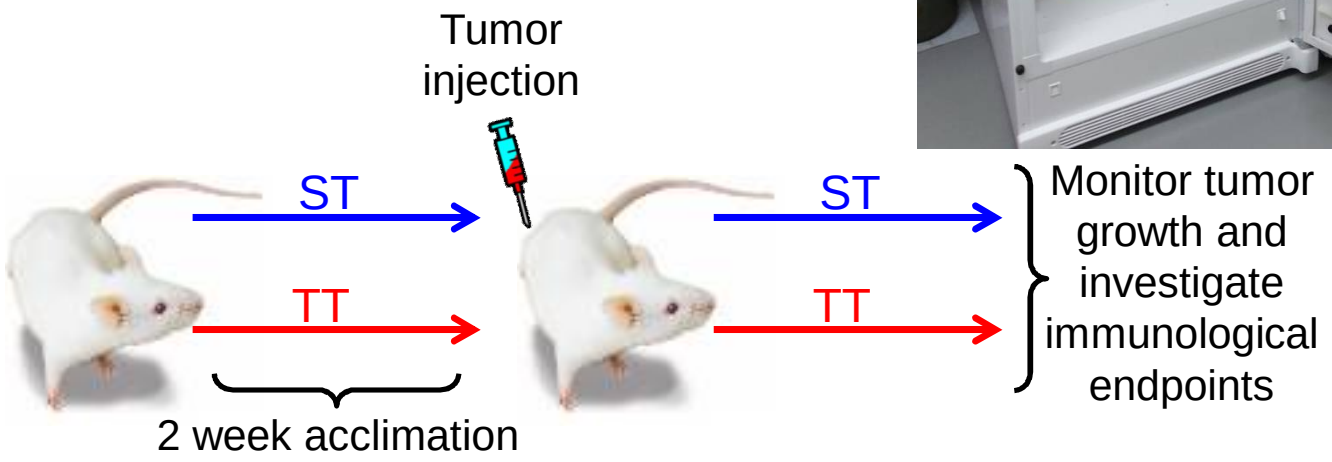


How is tumor growth influenced by ambient temperature?

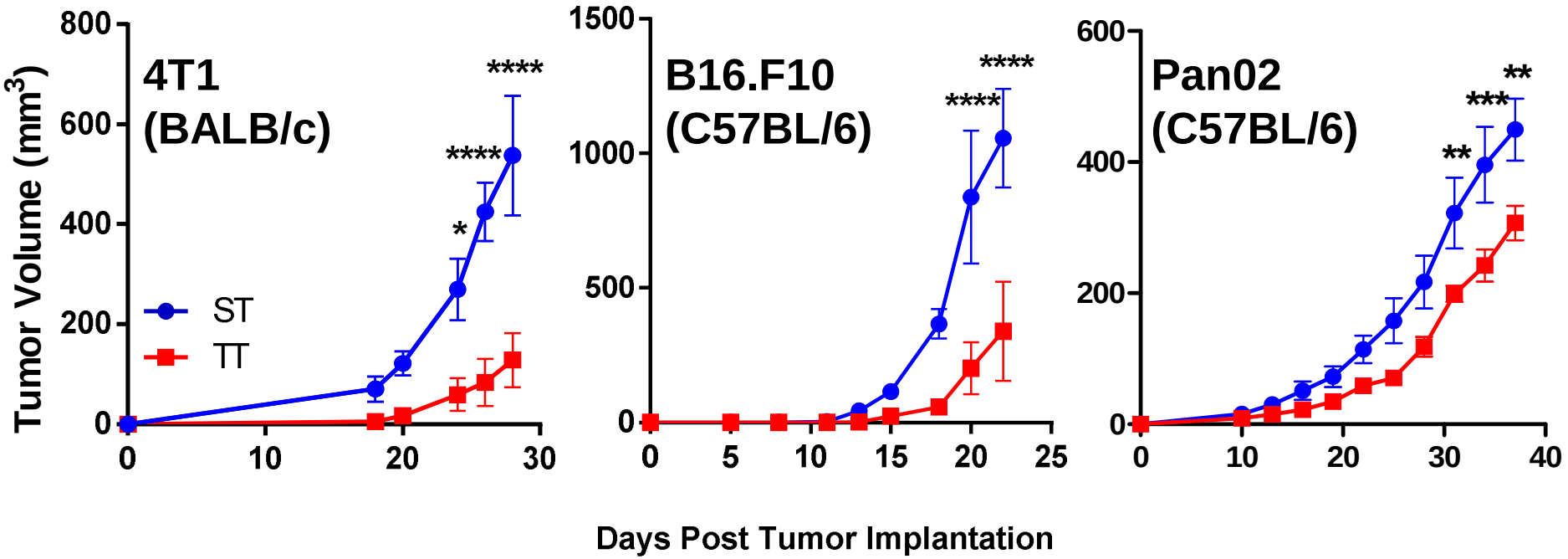
Standard Temperature (ST) → 22°C (~72°F)

Thermoneutral Temperature (TT) → 30°C (~86°F)

- Ambient temperature consistently maintained
- Humidity controlled with an air pump
- 12 hour light/dark cycle (6 AM – 6 PM)



Alleviating Cold Stress by Housing Mice at TT Significantly Improves Tumor Control

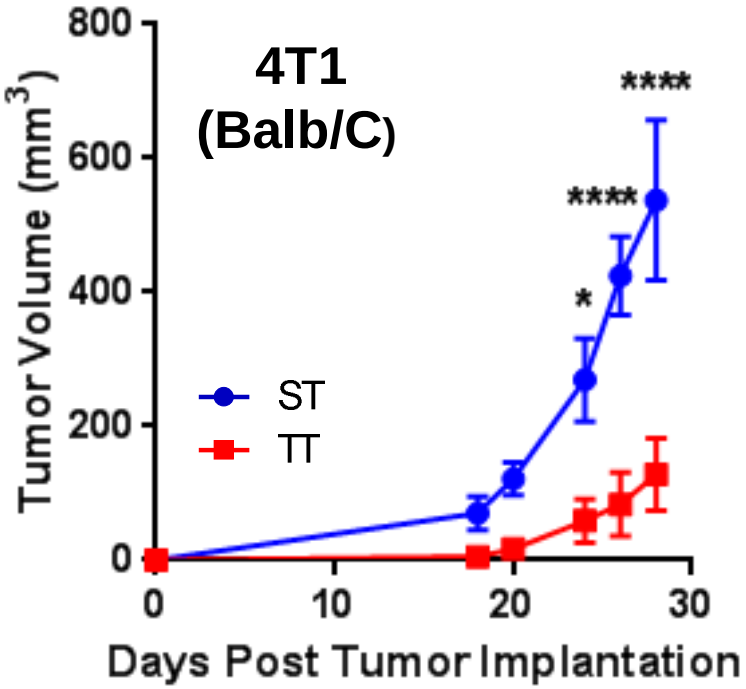


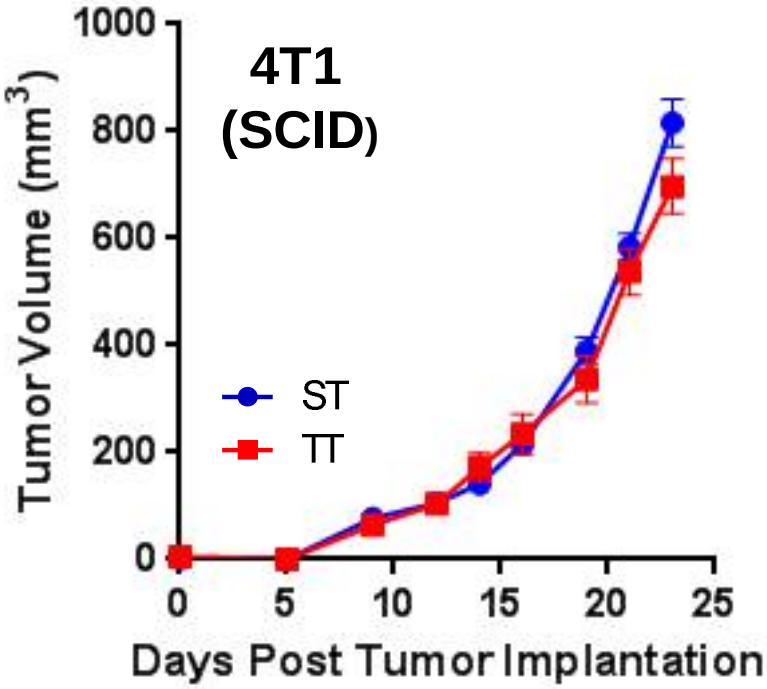
n=5-6/group

*p<0.05
** p<0.01
*** p<0.001
**** p<0.0001

Kokolus et al, *PNAS* 2013

Tumor growth is significantly slower in mice housed at thermoneutrality and this depends on the immune system

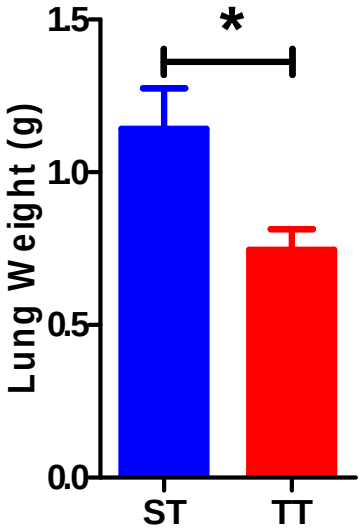


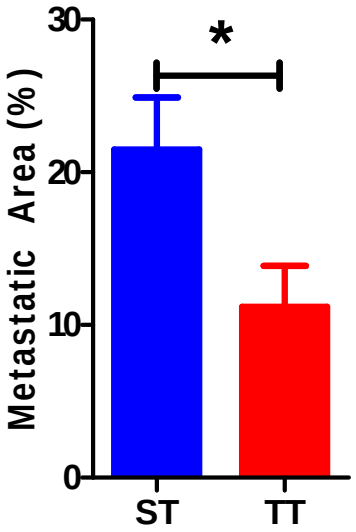


* $p < 0.05$
**** $p < 0.0001$

Kokolus et al, PNAS 2013

Mice Housed at TT Develop Fewer Metastastic Tumors (4T1 tumor model)

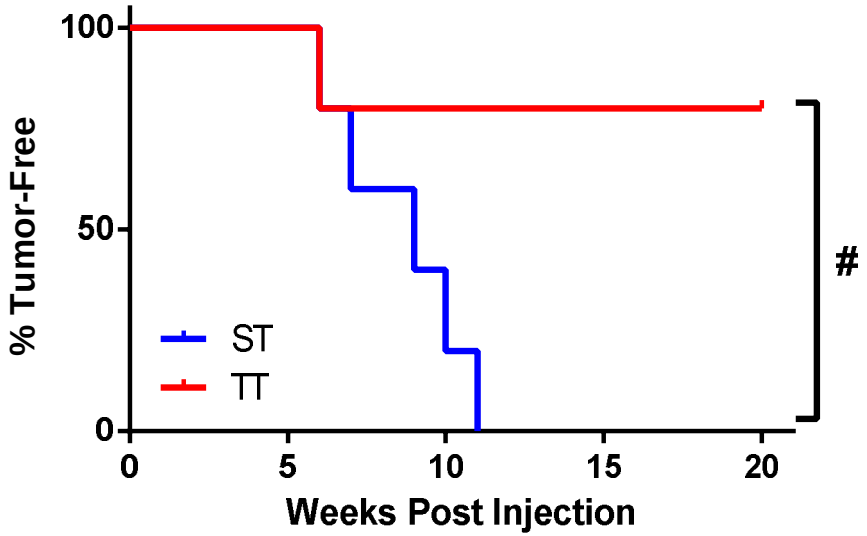




Standard Temperature **Thermoneutral Temperature**

Kokolus et al., *PNAS* 2013

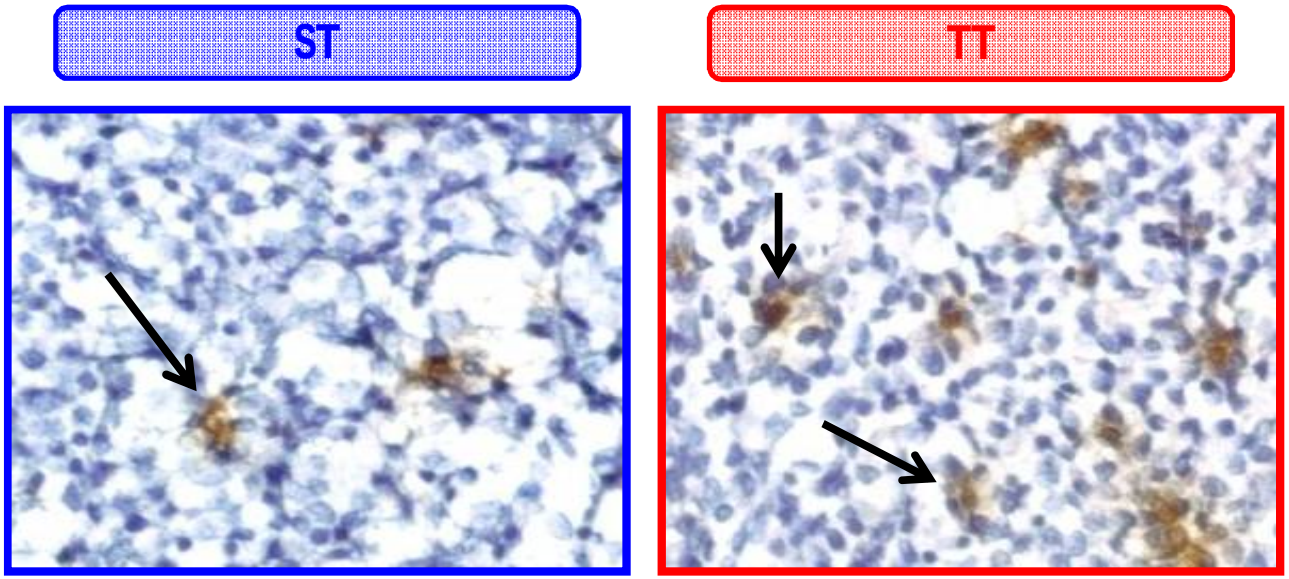
Carcinogen (methylcholanthrene) induced tumor formation is prevented or delayed at TT

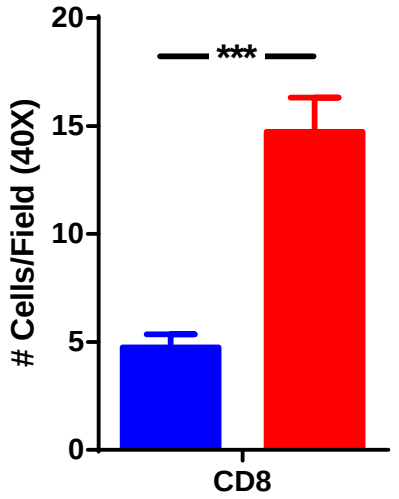


N = 5; # $p < 0.05$

Kokolus et al, PNAS 2013

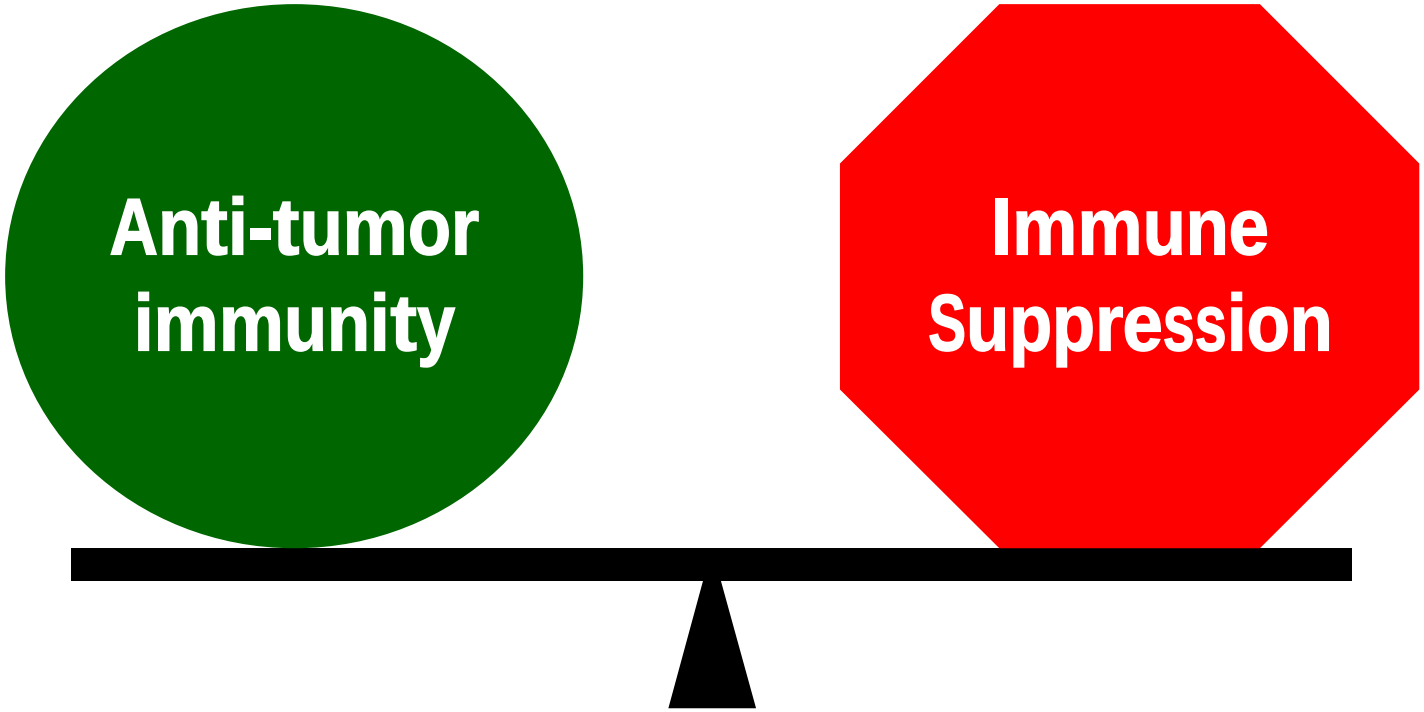
CD8+ T Cells in the Tumor Microenvironment are Increased at TT





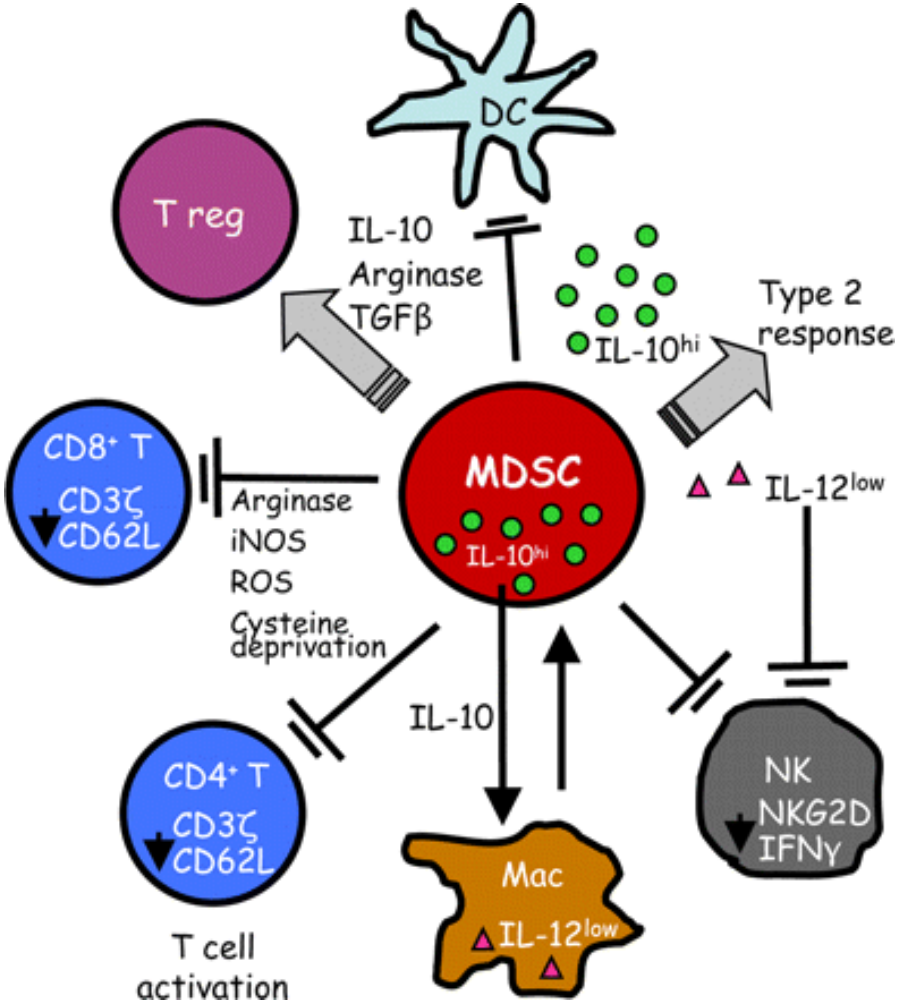
N = 5 - 6; * $p < 0.05$, *** $p < 0.001$

Kokolus et al.,- *PNAS* 2013



Are immunosuppressive cells impacted by cold stress?

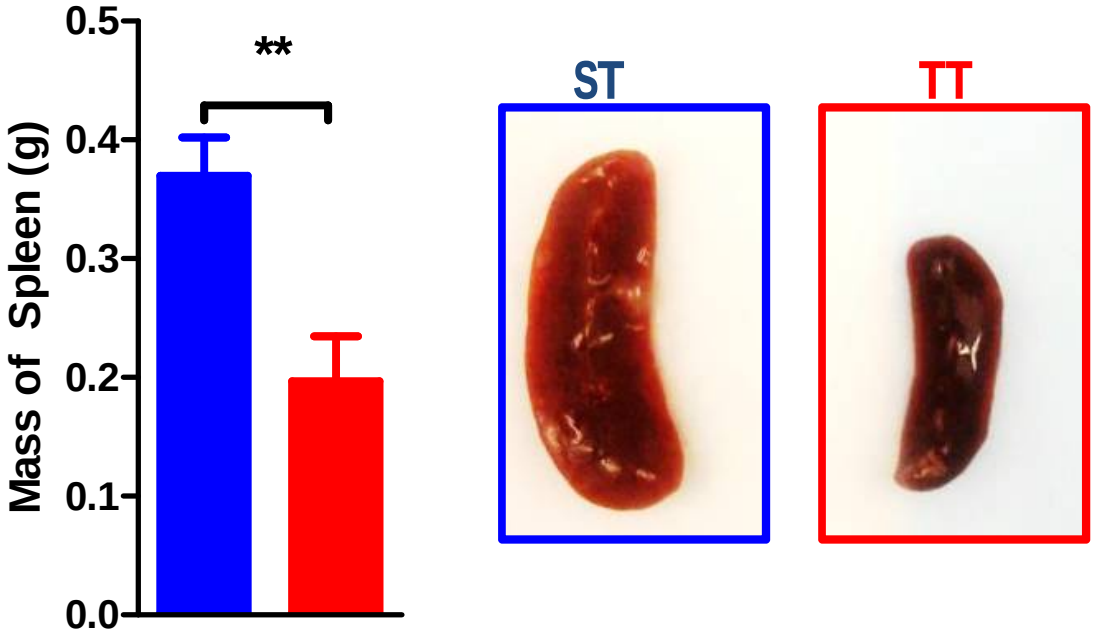
Myeloid derived suppressor cells suppress antitumor immunity through a variety of diverse mechanisms.



Ostrand-Rosenberg and Sinha 2009

Decreased Spleen Mass in Tumor Bearing Mice at TT

The spleen is a critical repository for MDSCs prior to their trafficking to the tumor microenvironment. (Cortez-Retamozo et al., 2012)

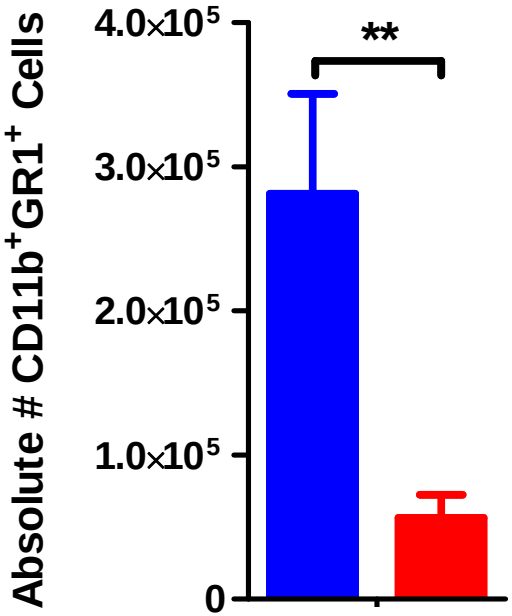


N=10/group

** p<0.01

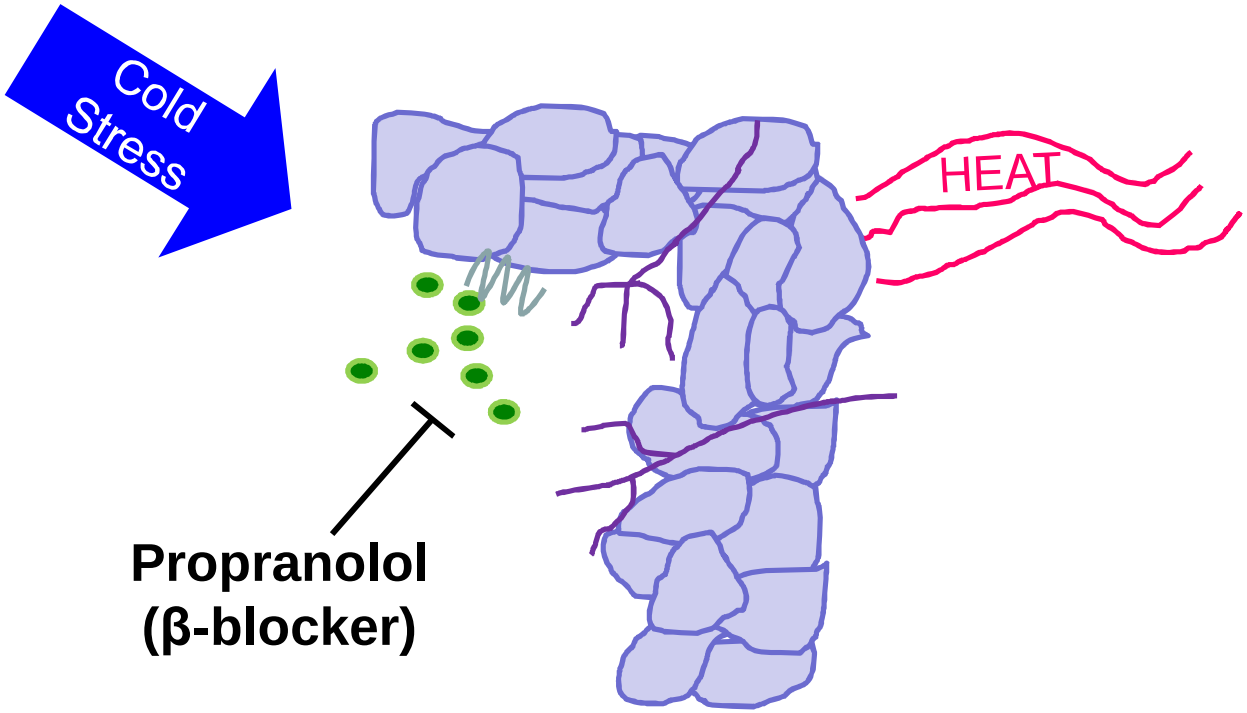
Significant Reduction in MDSC in Tumor Bearing Mice at TT

Flow Cytometry (Spleen)



Kokolus et al., PNAS 2013

Does blocking β-adrenergic signaling in mice at ST or TT impact tumor growth?

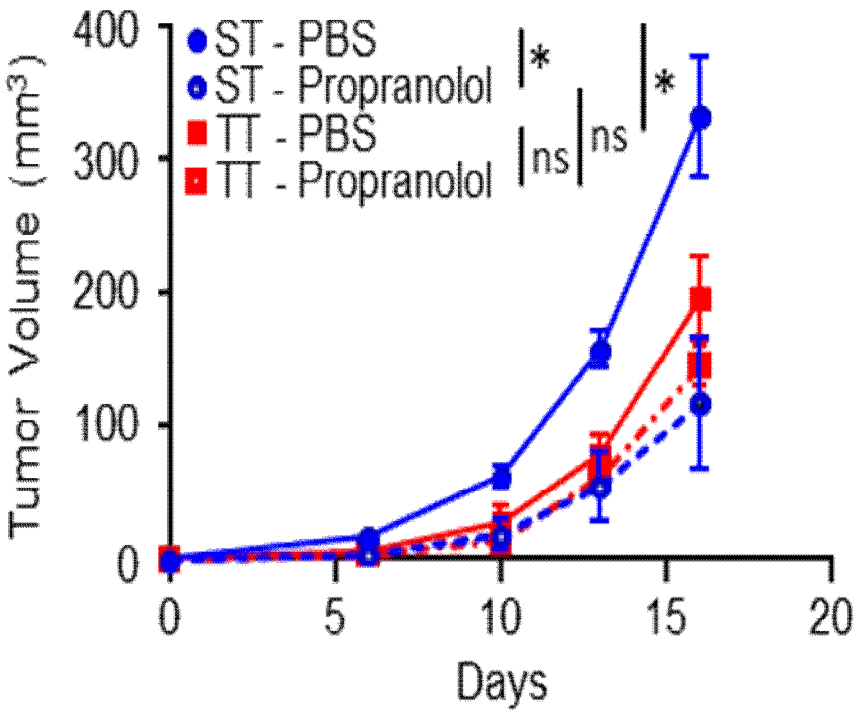


Propranolol-treatment converts the ST phenotype to that seen at TT

Effect depends upon presence of immune system!

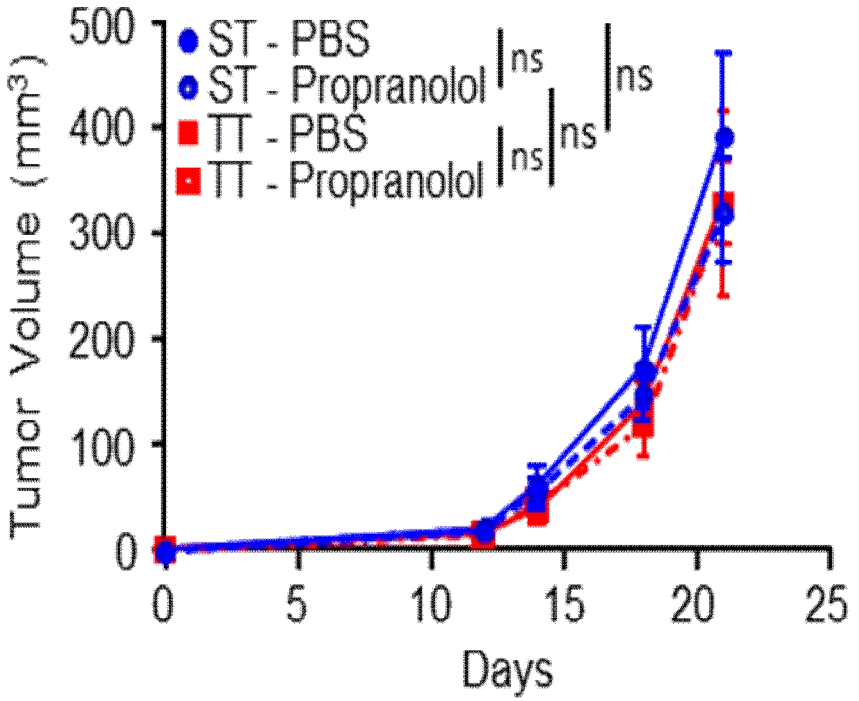
(A)

BALB/c - 4T1 Tumor



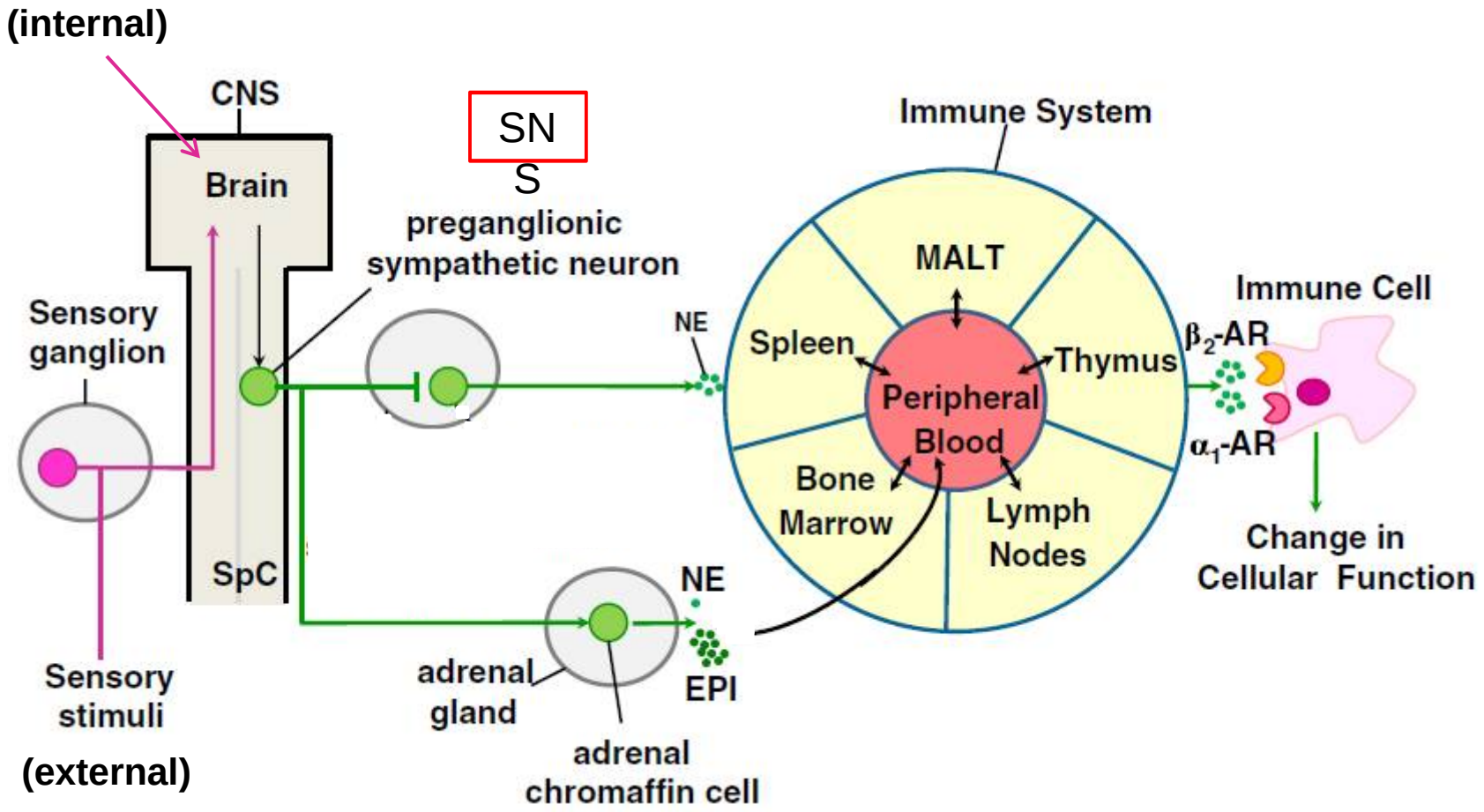
(B)

SCID - 4T1 Tumor



Mark Bucsek, Katie Kokolus

The sympathetic system can impact immune cells via mechanisms now being defined



Adapted from Bellinger DL and Lorton D, Autonom Neuro 2014

Conclusions: Ambient temperature impairs the anti-tumor immune response

- Chronic thermal stress results in reduced CD8+ T cell infiltration and increased Treg/MDSC
- Increased levels of NE in tumors
- Can be reversed by TT or β-blockers
- Causes environmentally induced resistance to cytotoxic therapies

- *Thermal damage may include the impact of chronic mild thermal stress*

Growing recognition of an ominous role for norepinephrine in tumor progression

- Adrenergic signaling protects ovarian tumor cells from anoikis-apoptosis due to detachment (Sood et al 2010; Cole & Sood, Clin Canc Res, 2012)

- NE increases VEGF, IL-6, and MMPs in ovarian and pancreatic cancers leading to increase metastasis. (Thakar et al, Nature Medicine 2006; Lutgendorf et al, Clinical Cancer Research 2003; Guo et al, Oncology Reports 2009)

- NE downregulates MHC class 1 and B7-1 on tumor cells leading to immune escape. (Wang et al, Plos One 2009)

- NE enhances prostate tumor survival by upregulating the expression of MAPK, and inactivating the apoptotic molecule, BAD. (Sastry, JBC 2007; Hassan et al, JCI 2012)

- Activation of β-adrenergic receptors promotes the formation of primary tumors and metastases. (Campbell et al PloS One 2012; Magnon et al Science 2013)

Thanks to:

Katie Kokolus, PhD (Postdoc with Todd Schell at Penn State Hershey)
Jason Eng, PhD (MD/PHD Student, now back in Medical School, SUNY Buffalo
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Mark Bucsek (MD/PhD student)

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Scott Abrams, PhD
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Lloyd Foundation for Melanoma Research