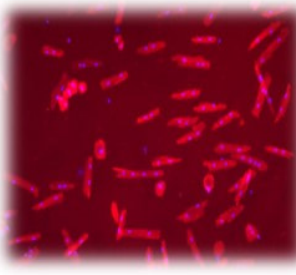




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WHBA, Inc.**

**8th International Summer School in
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May 17-24, 2019
Athens & Neo Itilo, Laconia – Greece
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Disruption of a self-amplifying catecholamine loop reduces cytokine release syndrome

Verena Staedtke^{1,2,7*}, ren-Yuan Bai^{3,7*}, Kibem Kim¹, Martin Darvas⁴, Marco L. Davila⁵, Gregory J. riggins³, Paul B. rothman⁶,
Nickolas Papadopoulos¹, Kenneth W. Kinzler¹, Bert Vogelstein^{1*} & Shubin Zhou^{1*}

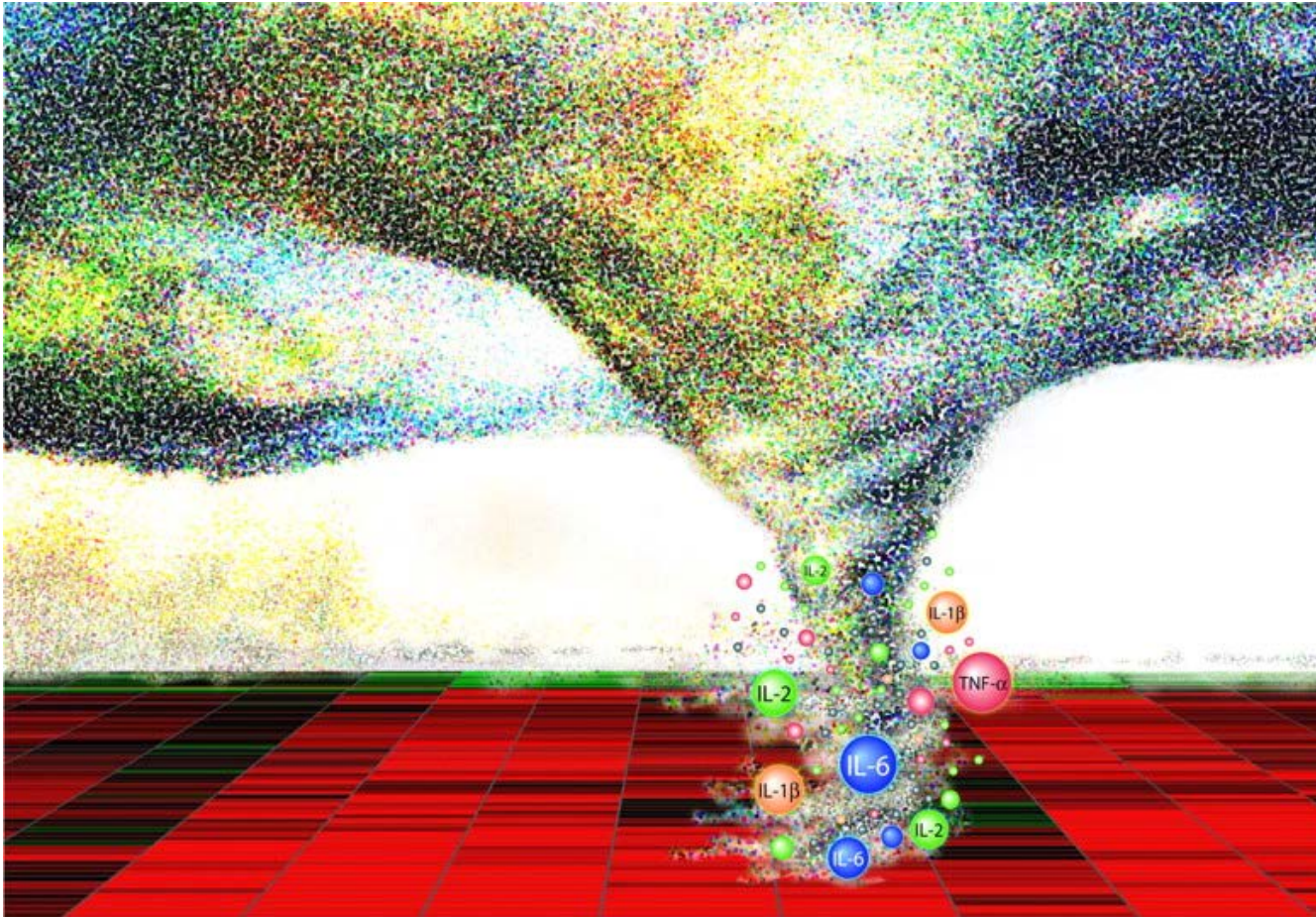
Instructor: Nicki Panoskaltsis

Students: Chrysanthi Mantsiou

Despina Christina Pavlidou

Eirini Papamanoli

What is cytokine release syndrome (CRS)?



a harmful systemic
hyperactivated
immune state



- cardiovascular collapse
- multiple organ dysfunction
- death

Causes of CRS

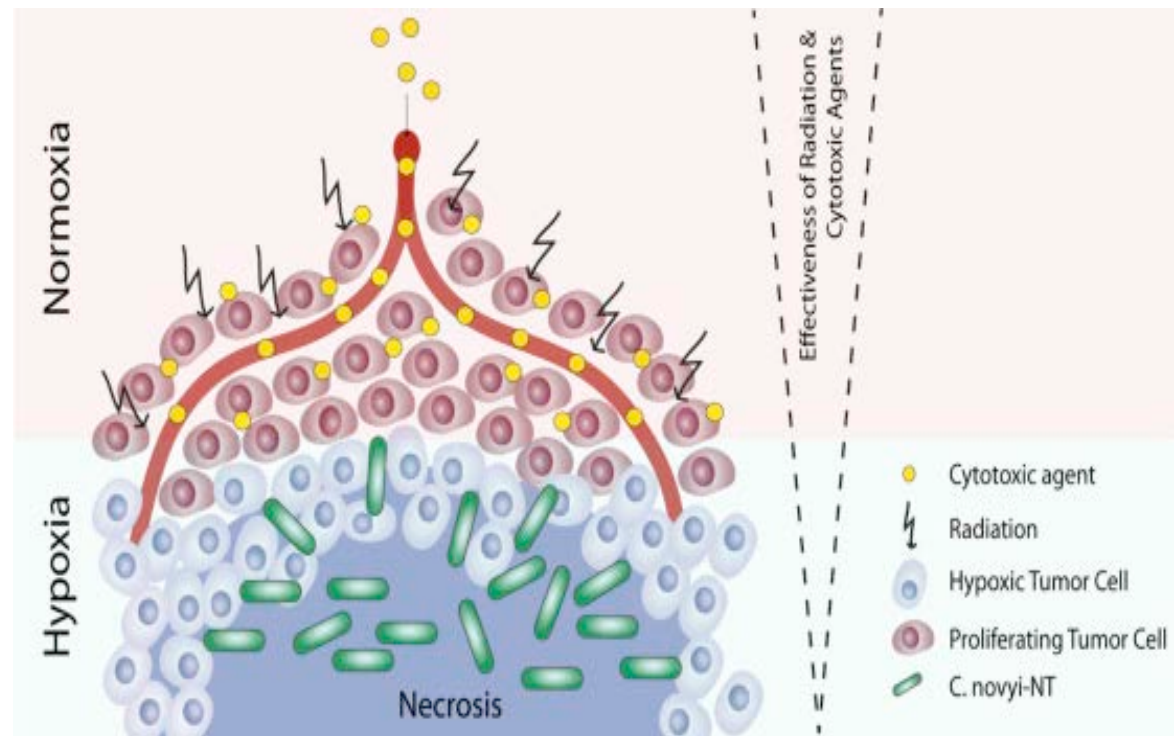


- **naturally occurring pathogens**
- **biotherapeutic agents**
 - oncolytic viruses and bacteria
 - recombinant lymphokines
 - natural and bispecific antibodies
 - T cells designed to kill cancer cells

How did the present study begin?

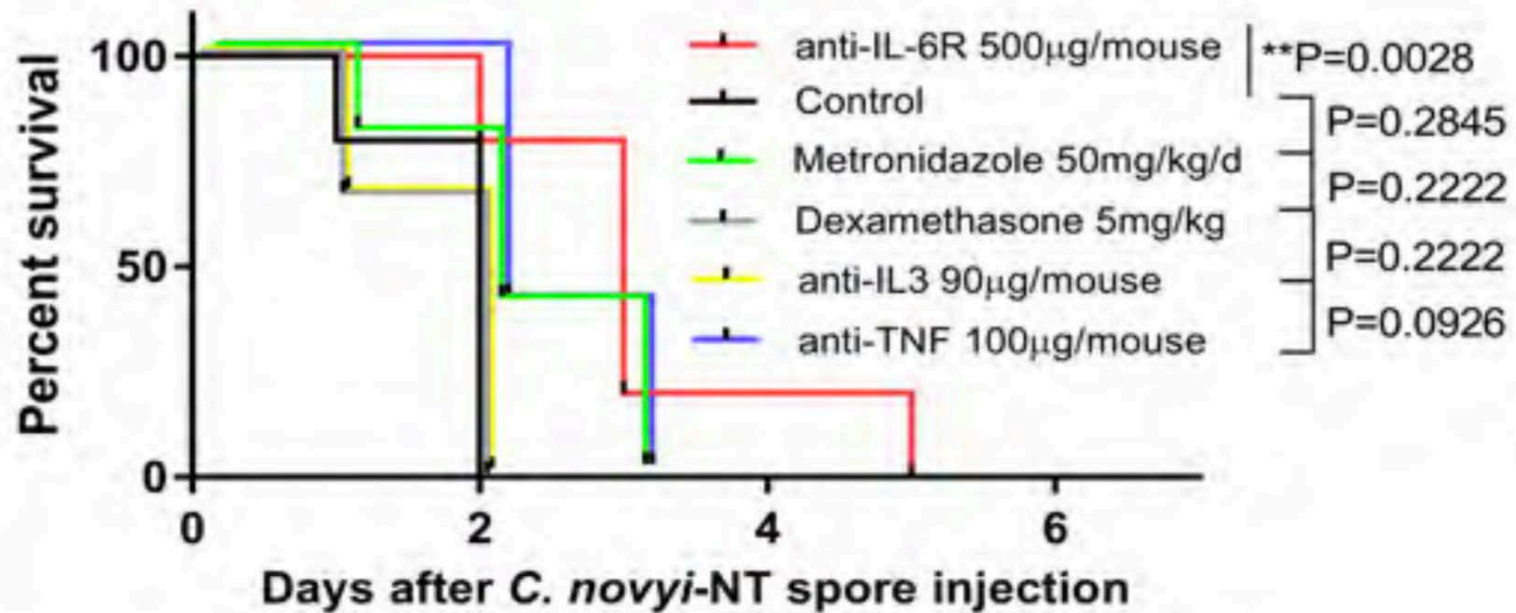
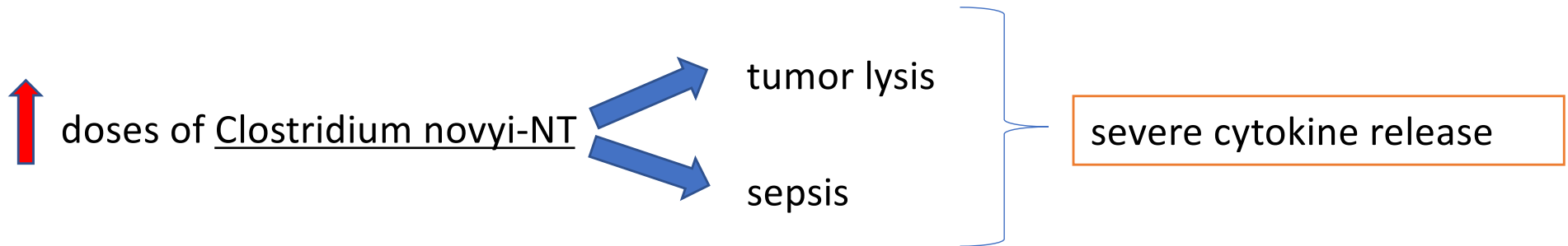
Clostridium novyi-NT

- ✓ Anaerobic
- ✓ Spore-forming
- ✓ Gram +



Shibin Zou et al, Genes and Diseases, 2016

But...



And then... ANP

ANP= Atrial Natriuretic Peptide

- released by cardiac cells
- regulates fluid and electrolyte homeostasis

Inhibition of I κ B kinase



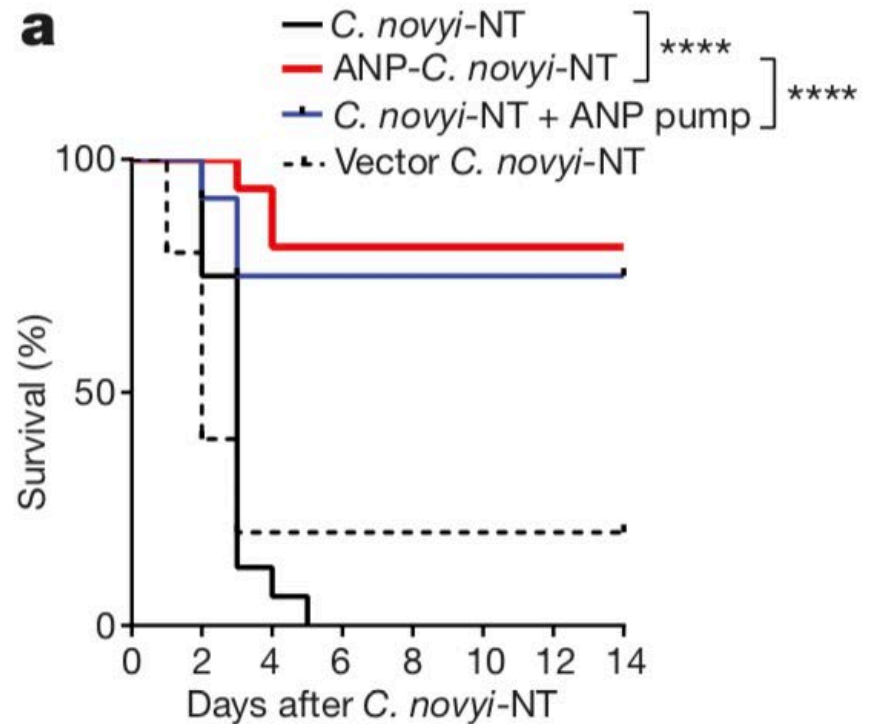
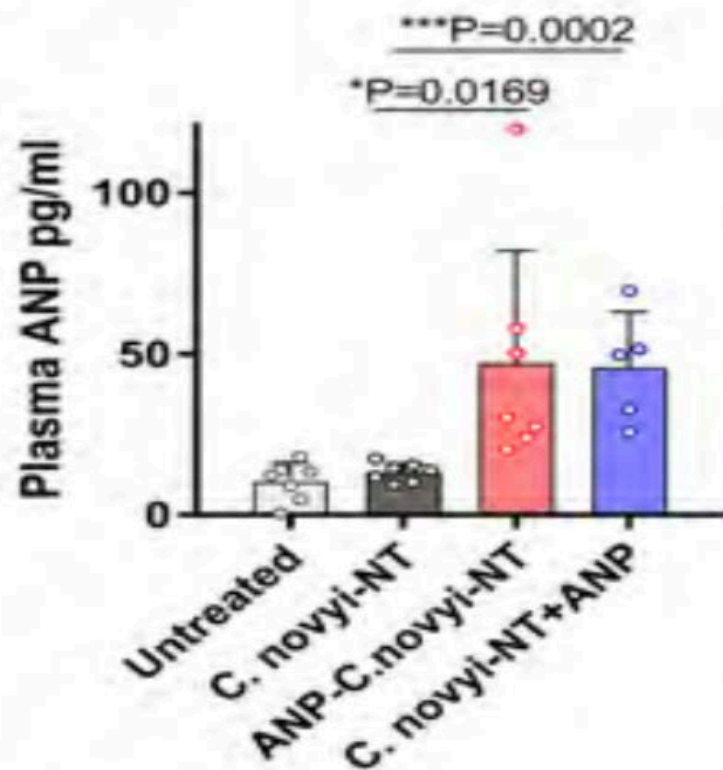
Not only!



They engineered *C. novyi*-NT to express and secrete ANP by stably integrating an expression cassette of ANP with a signal peptide into the *C. novyi*-NT genome using the group II intron targeting.

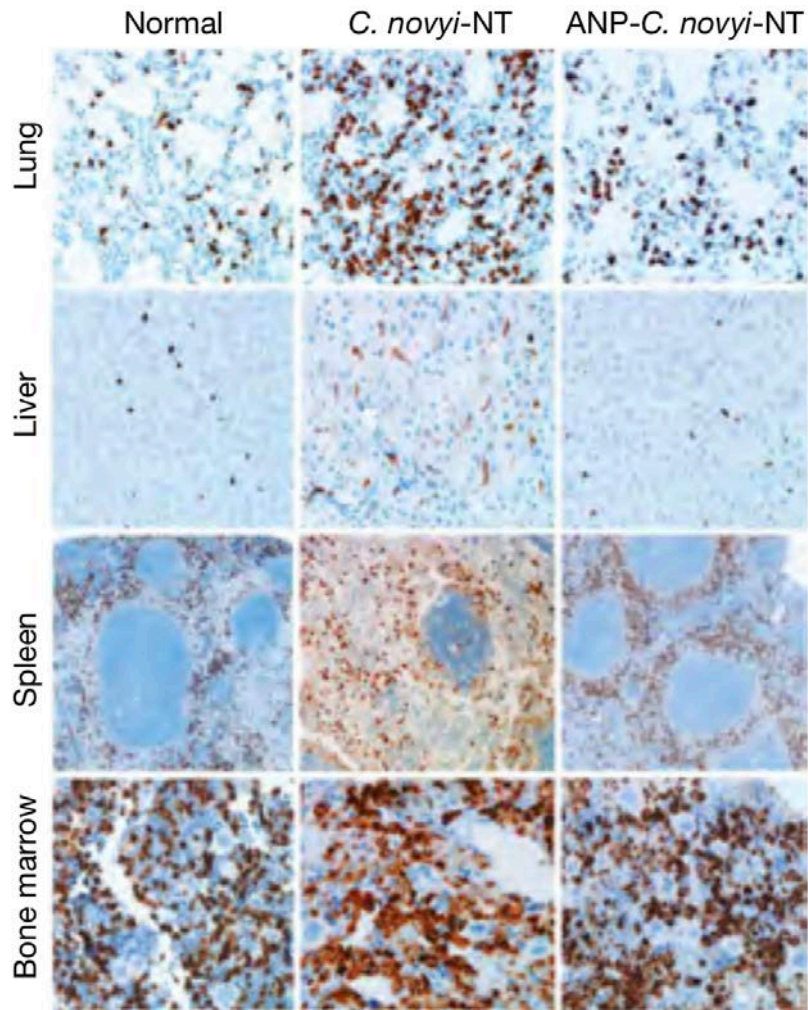
How did they proceed?

One dose of ANP-*C. novyi*-NT spores injected into subcutaneously implanted CT26 tumours resulted in robust germination and tumor regression.

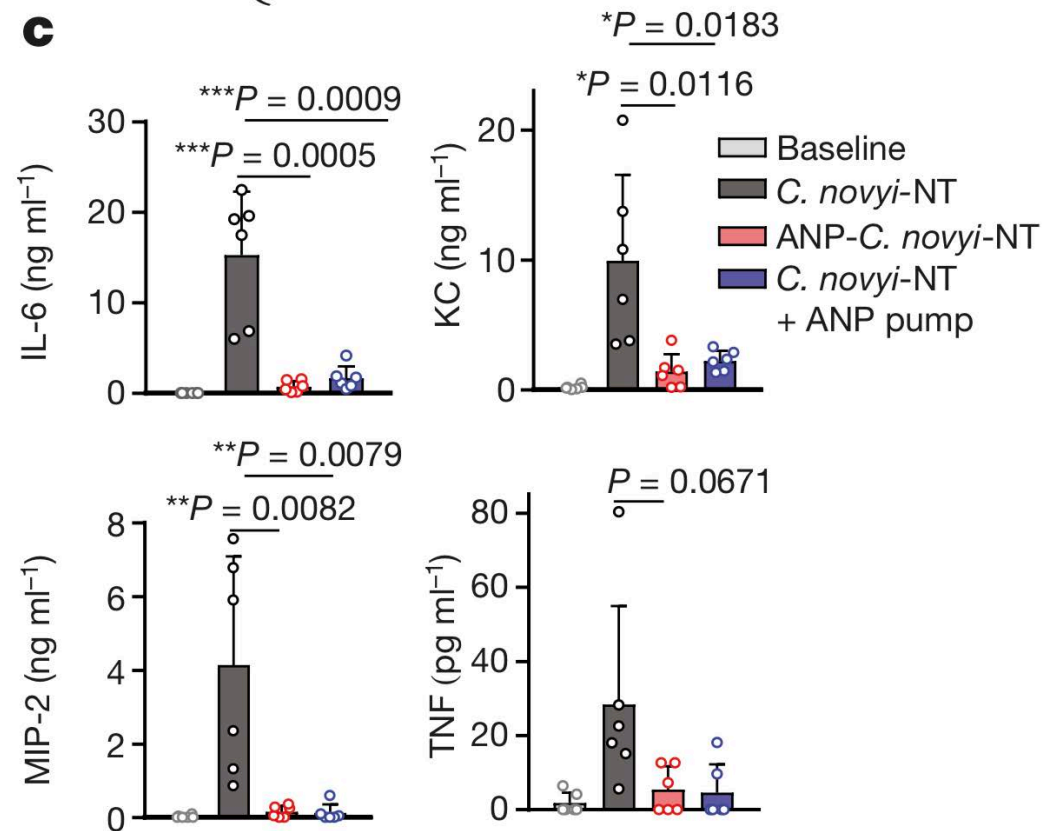


Mice injected with ANP-C. *novyi*-NT exhibited a noticeable reduction in tissue damage and inflammation

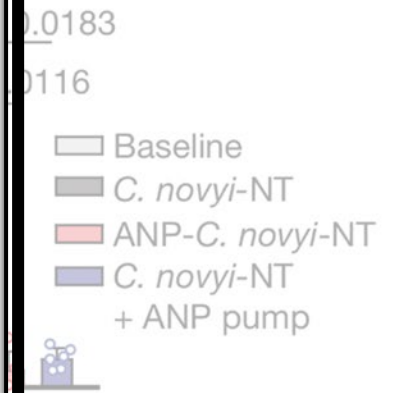
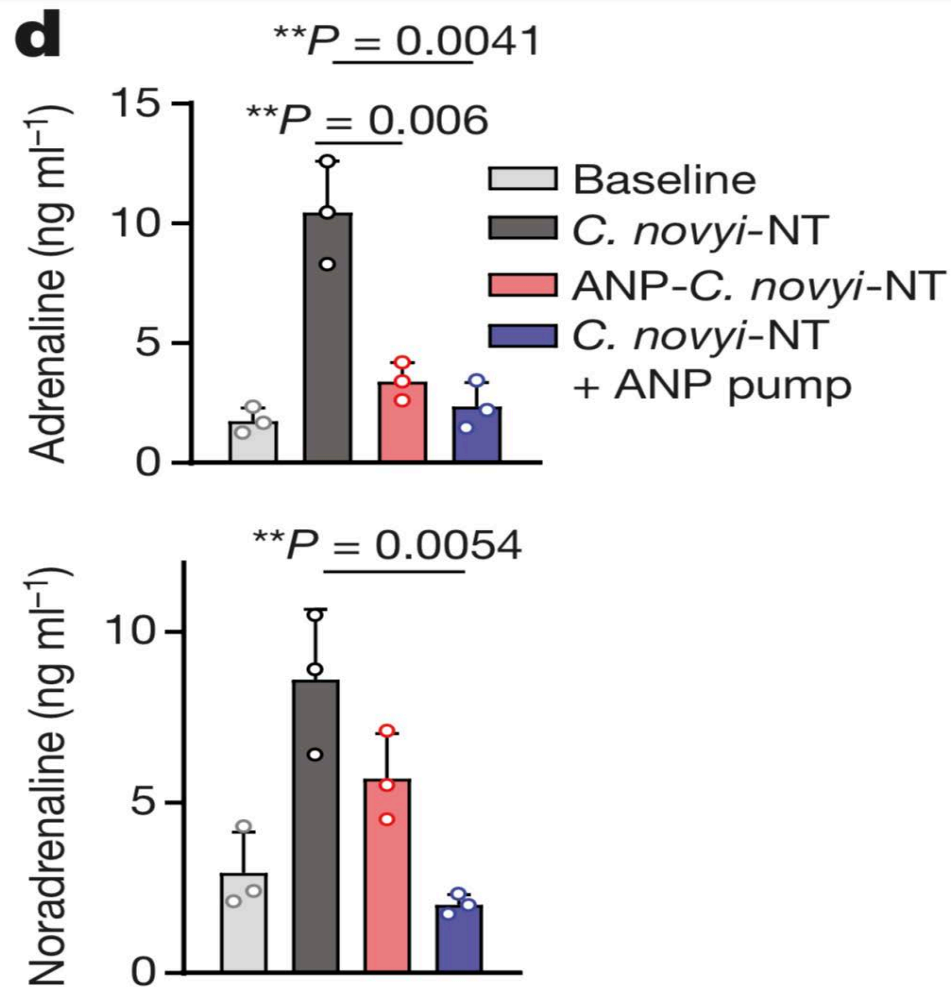
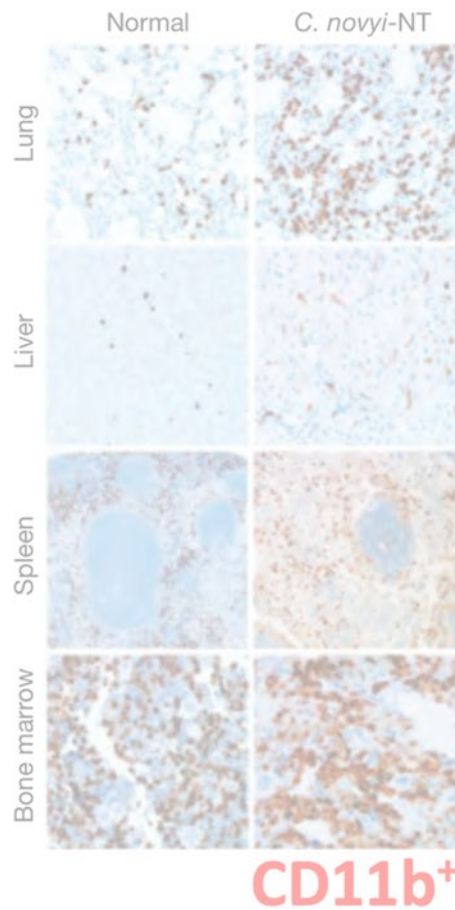
CD11b⁺



c



Mice injected with ANP-*C. novyi*-NT exhibited a noticeable reduction in tissue damage and inflammation

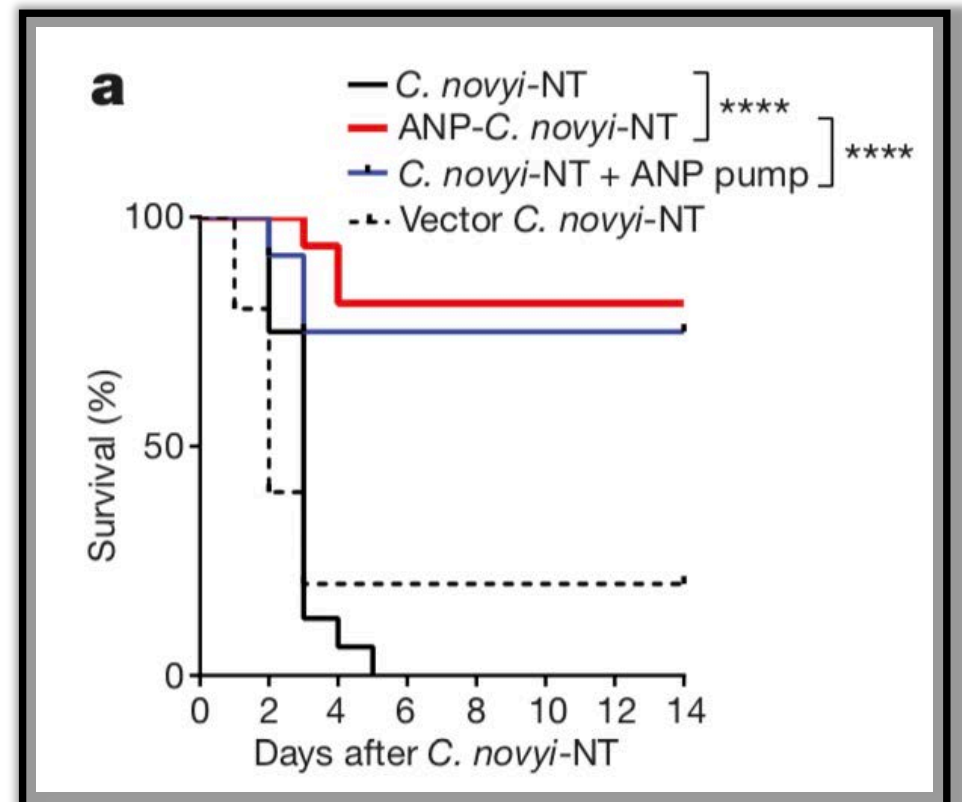


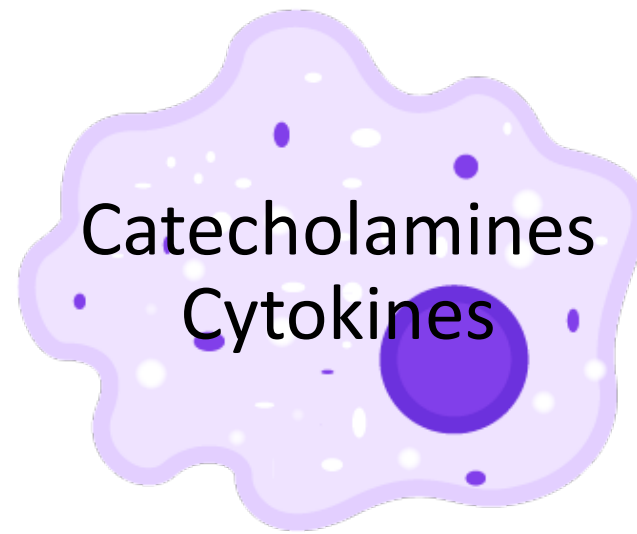
0.0671



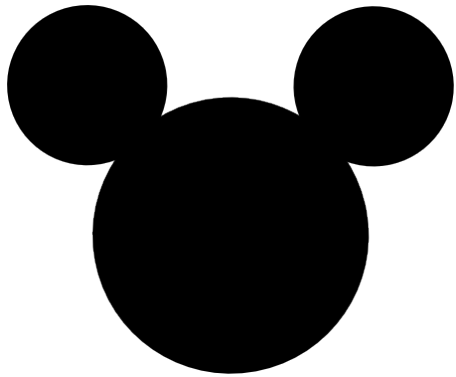
Is the protective effect due to expression of ANP?

They used **ANP-releasing osmotic pumps** implanted subcutaneously into mice before *C. novyi*-NT treatment.





Which catecholamine?



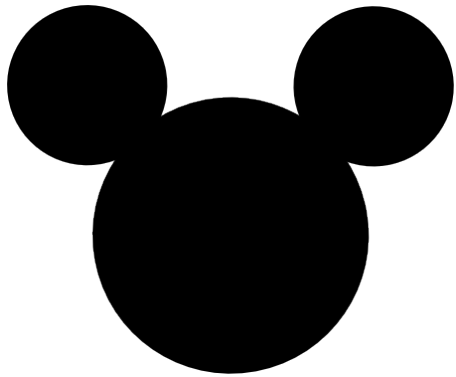
BALB/c mice

+ LPS

+

1. Adrenaline
2. Noradrenaline
3. Dopamine

Which catecholamine?



BALB/c mice

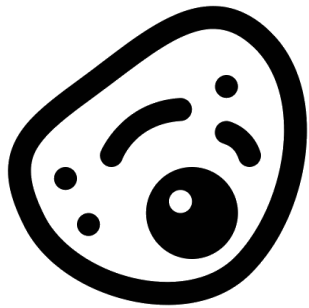
+ LPS

+

1. Adrenaline
2. Noradrenaline
3. Dopamine

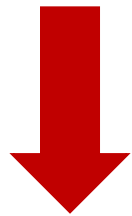
1. Exacerbated disease
2. Increased mortality
3. Decreased Il-6, TNF, KC

Role of ANP

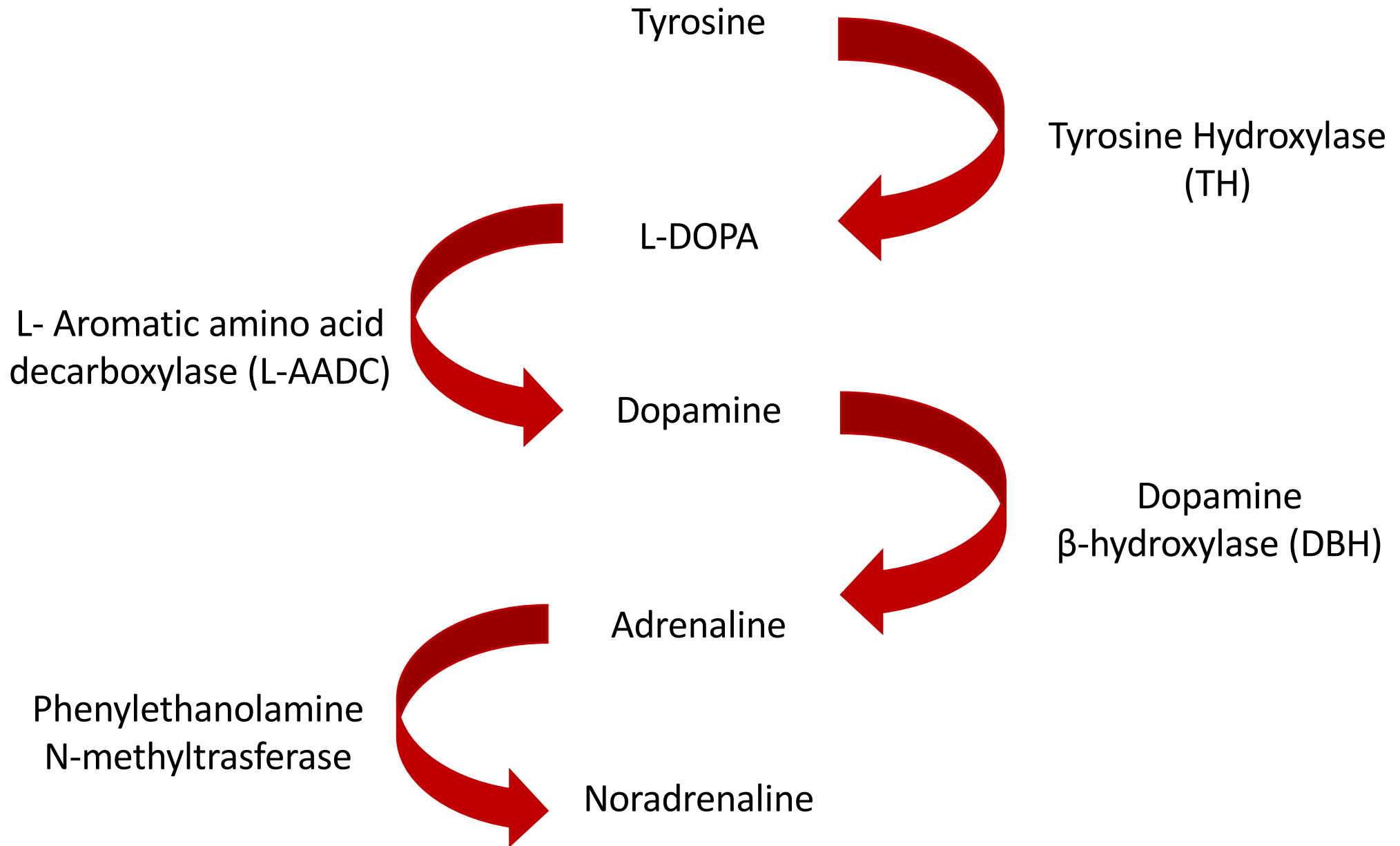


+ LPS + ANP

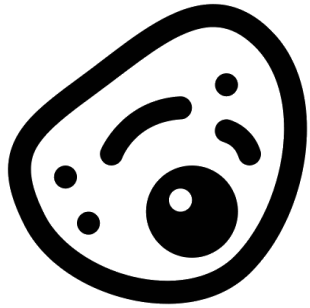
+ Adrenaline



IL-6, TNF, MIP-2, KC

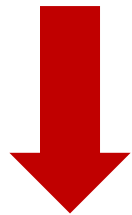


Role of Metirosine



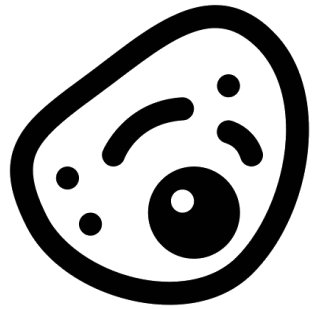
+ LPS + MTR

+ Adrenaline



catecholamines
cytokines

To confirm...

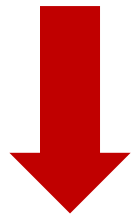


+

LPS

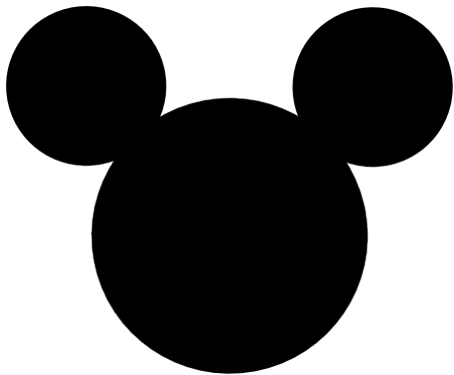
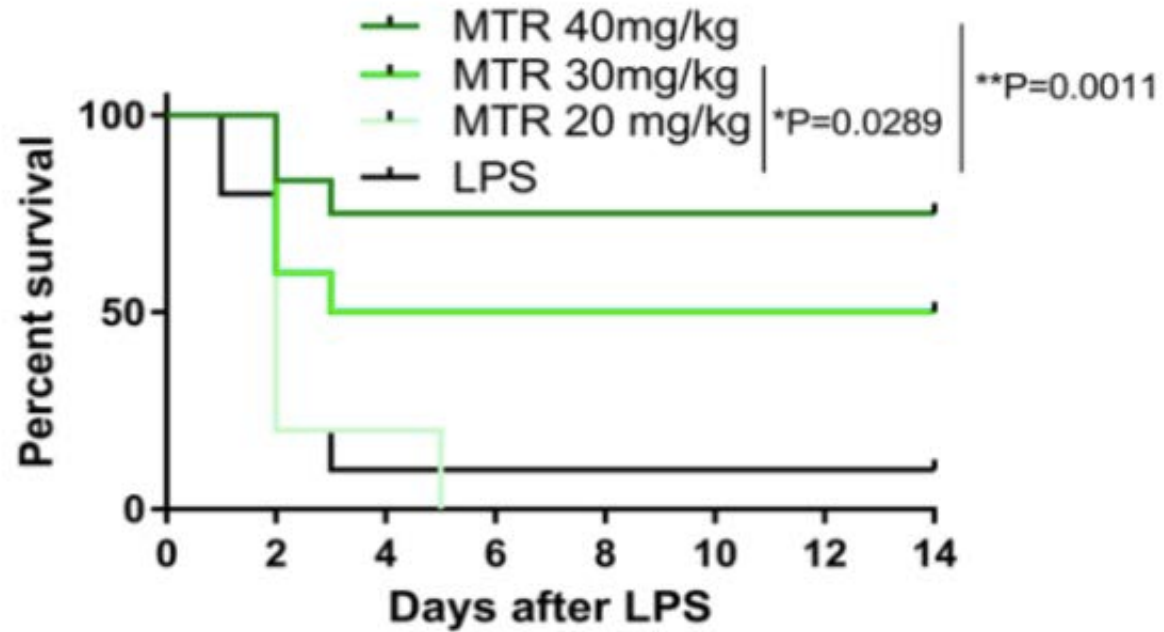
+

Deletion of
Th gene



catecholamines
cytokines

a



BALB/c mice

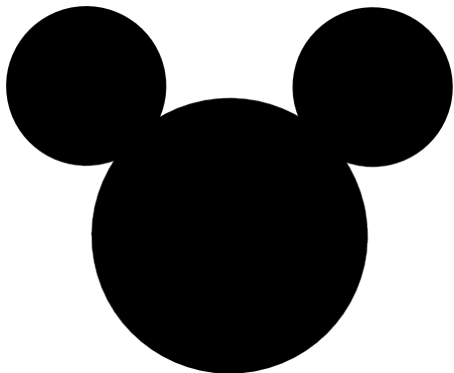
+

MTR

+

LPS

75%



+

LPS

10%

Which receptors?

$\alpha 1$

prazosin

$\alpha 2$

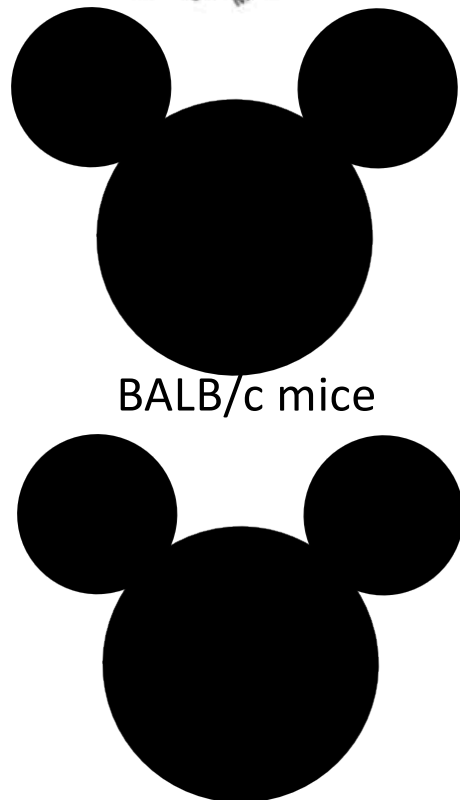
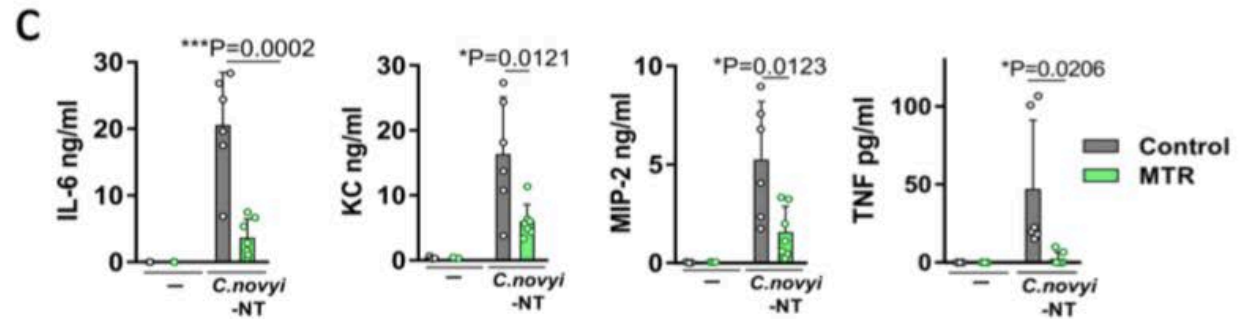
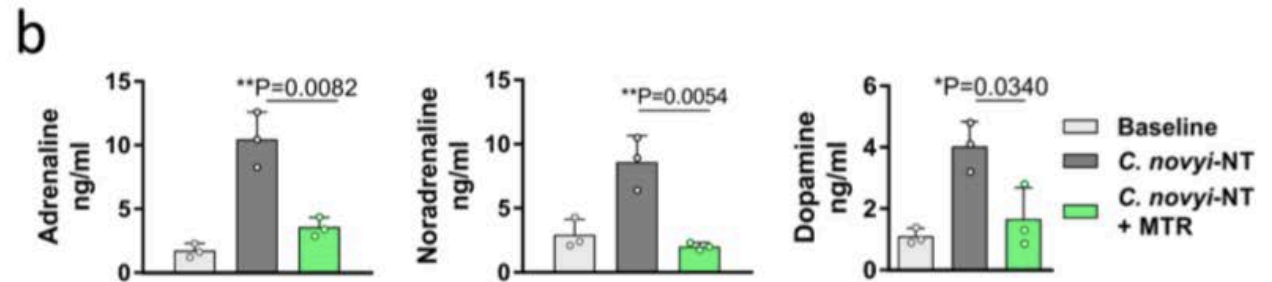
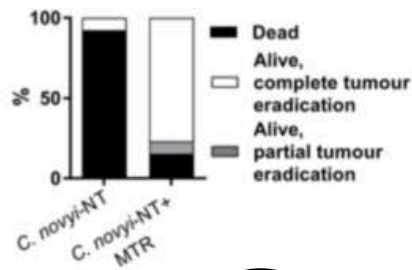
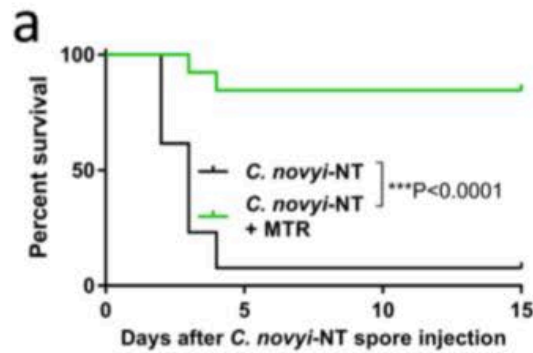
RX 821002

$\beta 1$

metoprolol

$\beta 2$

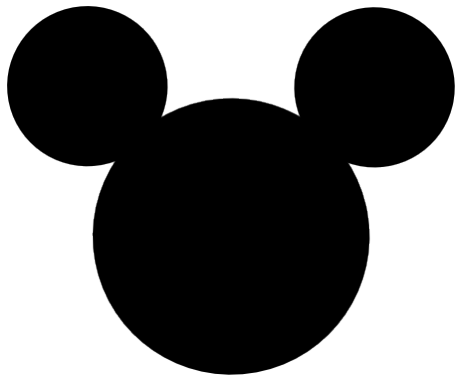
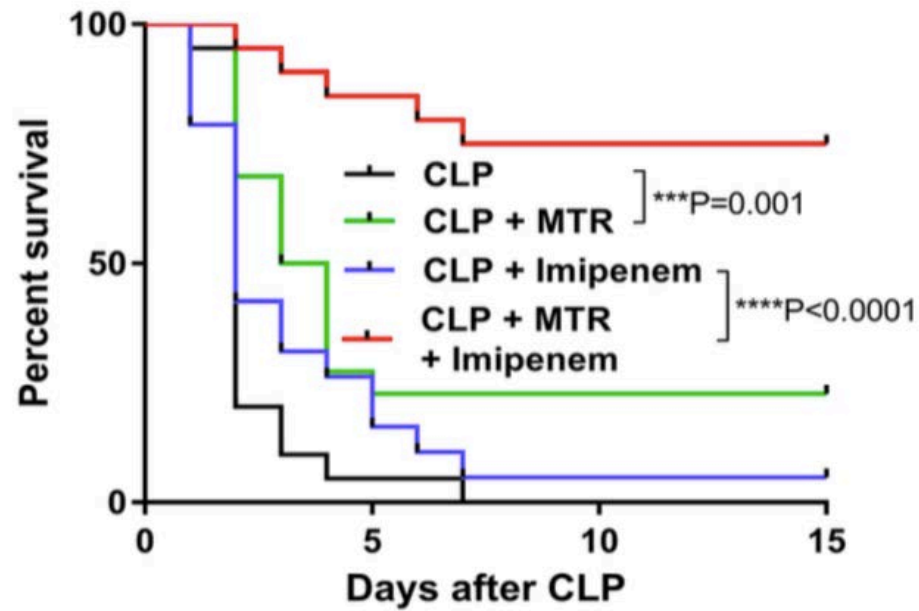
ICI 118551



+ MTR + *C. novyi*-NT 85%

+ *C. novyi*-NT 8%

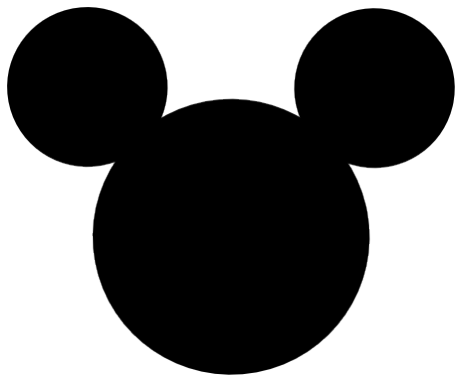
d



BALB/c mice

+ MTR + CLP

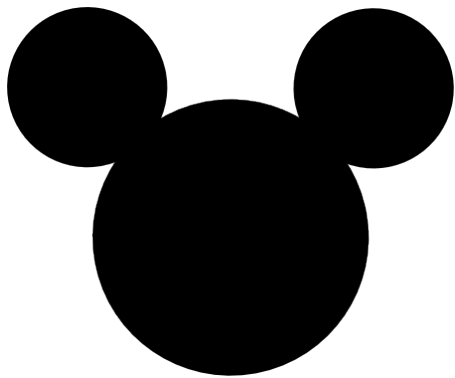
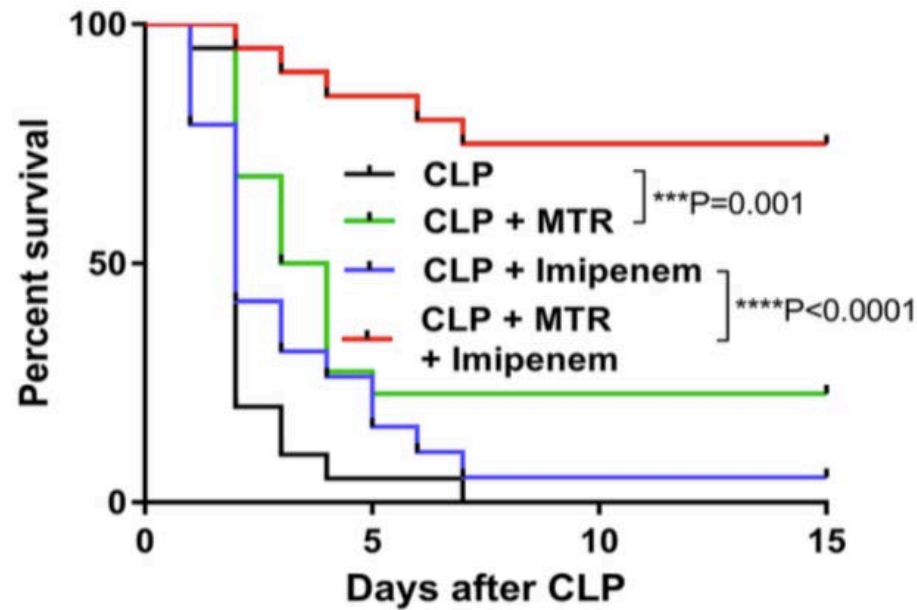
22%



+ CLP

0%

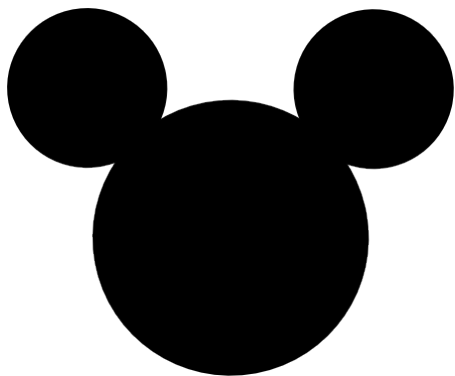
d



BALB/c mice

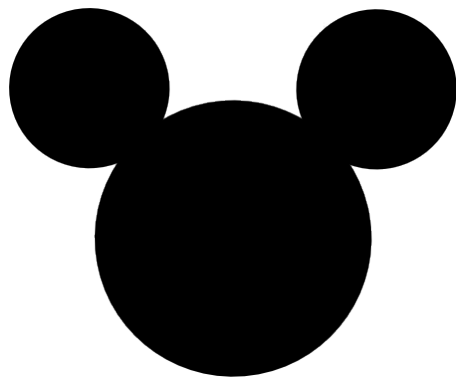
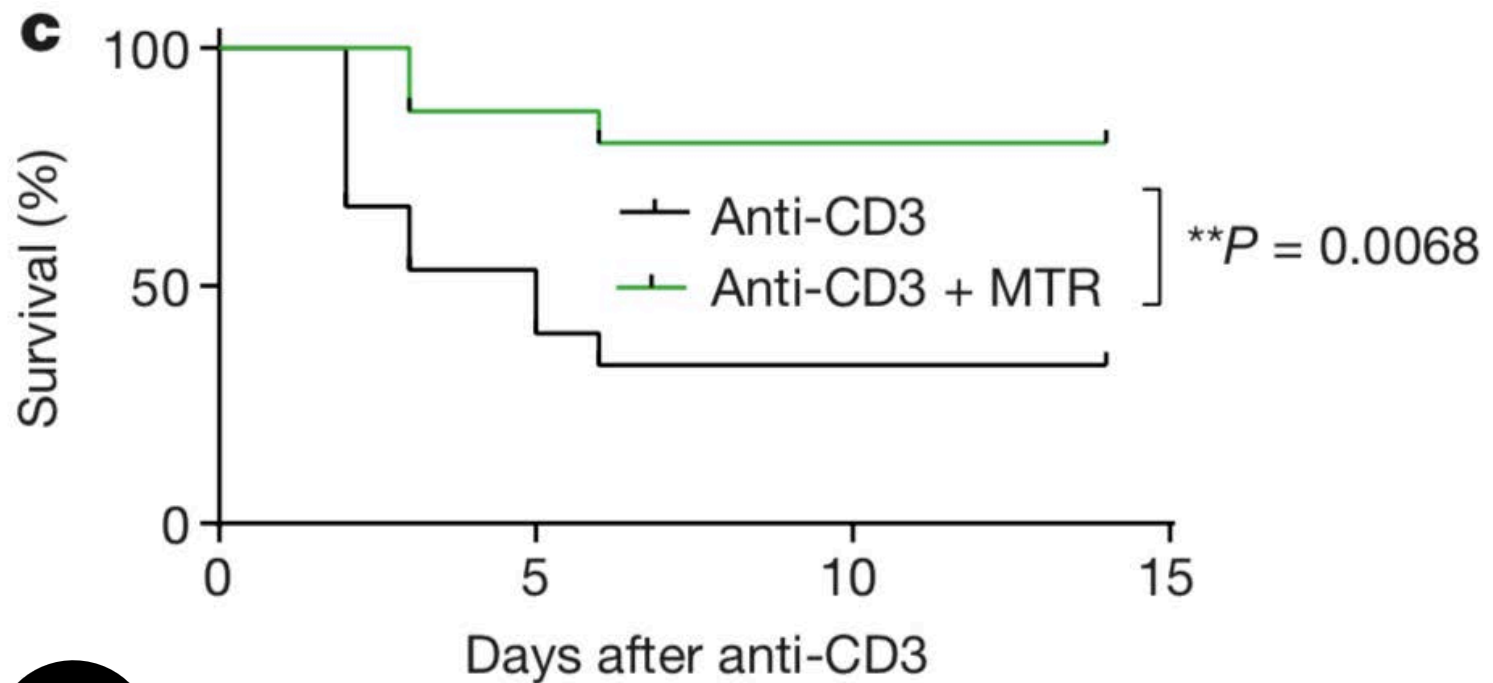
+ MTR + CLP
+ imipenem

66%



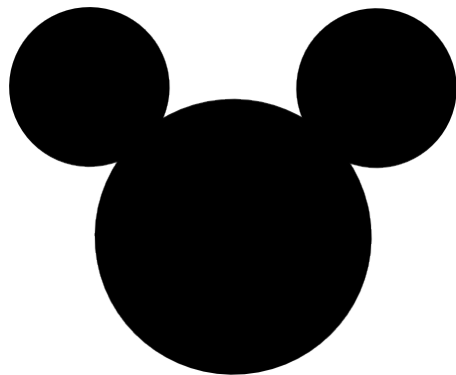
+ CLP
+ imipenem

10%



BALB/c mice

Anti-mouse
+ CD3 antibody 1452C11 + MTR



Anti-mouse
+ CD3 antibody 1452C11

Do CAR T-cells induce the same reaction?



But wait...

What are CAR T-cells??

- Genetically engineered to produce an artificial T-cell receptor
- **Chimaeric antigen receptors** – synthetic molecules that don't naturally exist
- Why chimaeric?
 1. T-cell functions
 2. New antigen-recognizing factors

CAR-T cell manufacturing

1. *the chimeric antigen receptor*
2. *the correct subset of T-cell markers*

This enables positive identification of the product and discrimination against untransduced T-cells and undesirable cell types that have been transduced with the CAR⁹.

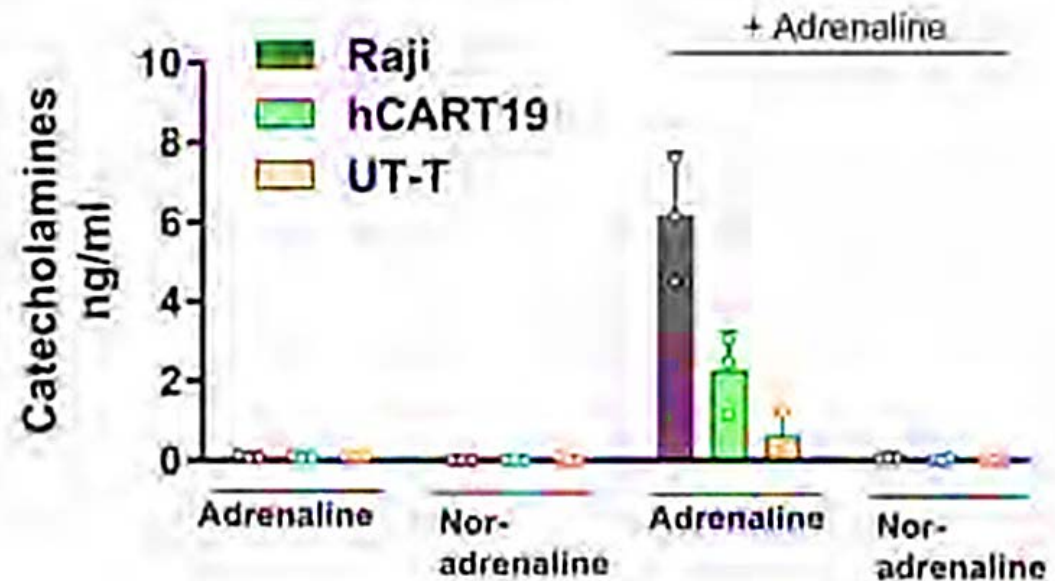
Do CARTs generate catecholamines?

human Burkitt's lymphoma-derived CD19+ Raji cells

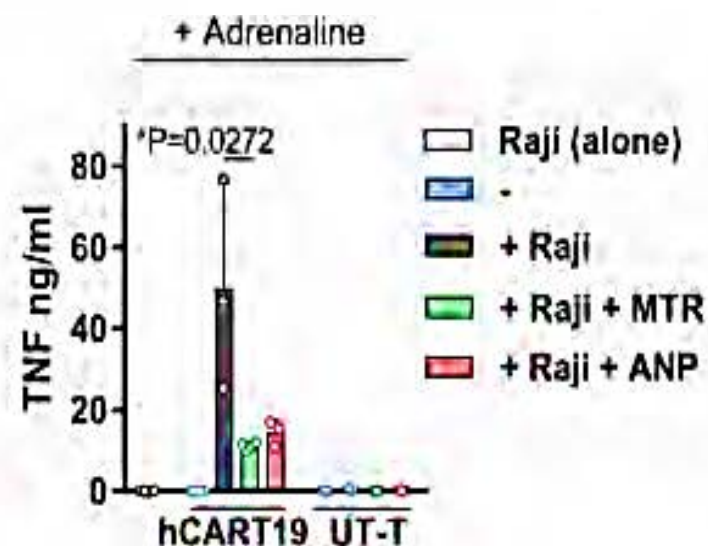
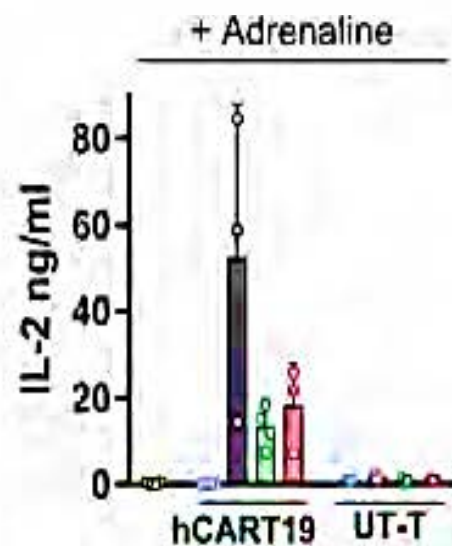
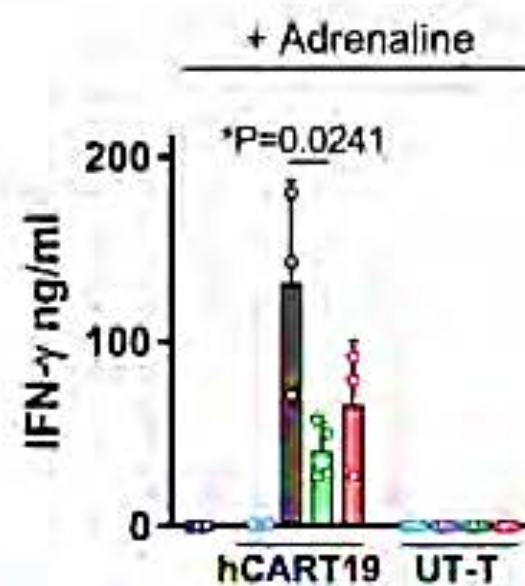
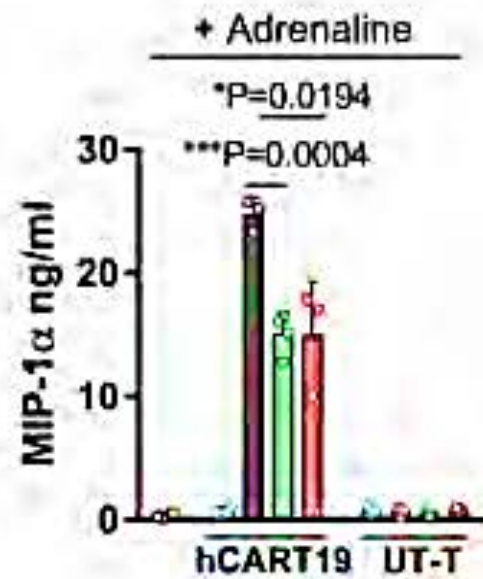
+ *in vitro*

hCART19 cells

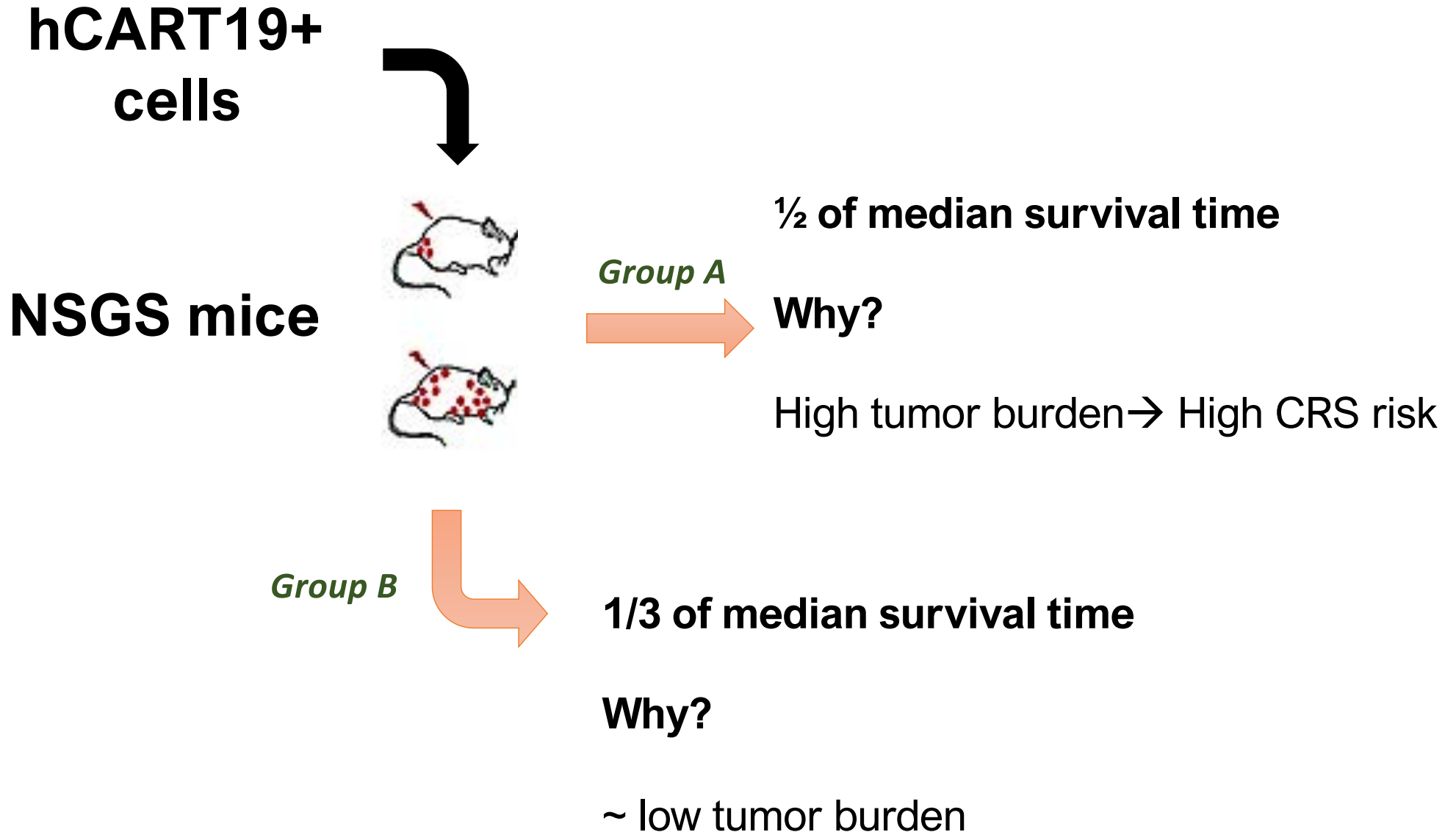
Release of
catecholamines and
cytokines



MTR and ANP abated the reaction



What was the *in vivo* result?



What was the *in vivo* result?

Group A

Premature death

Excessive catecholamine levels

Excessive cytokine levels

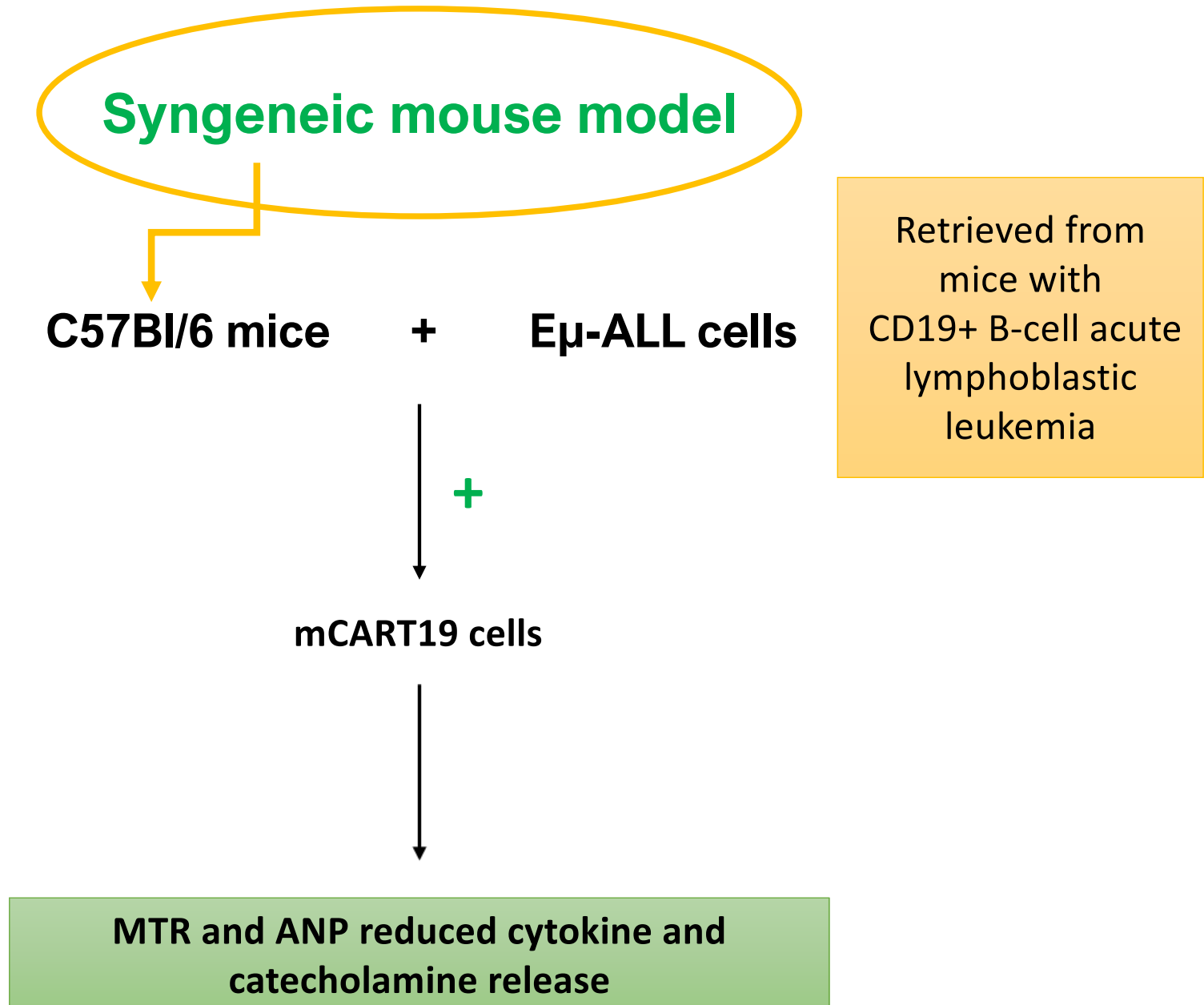
Group B

Substantial anti-tumor effect

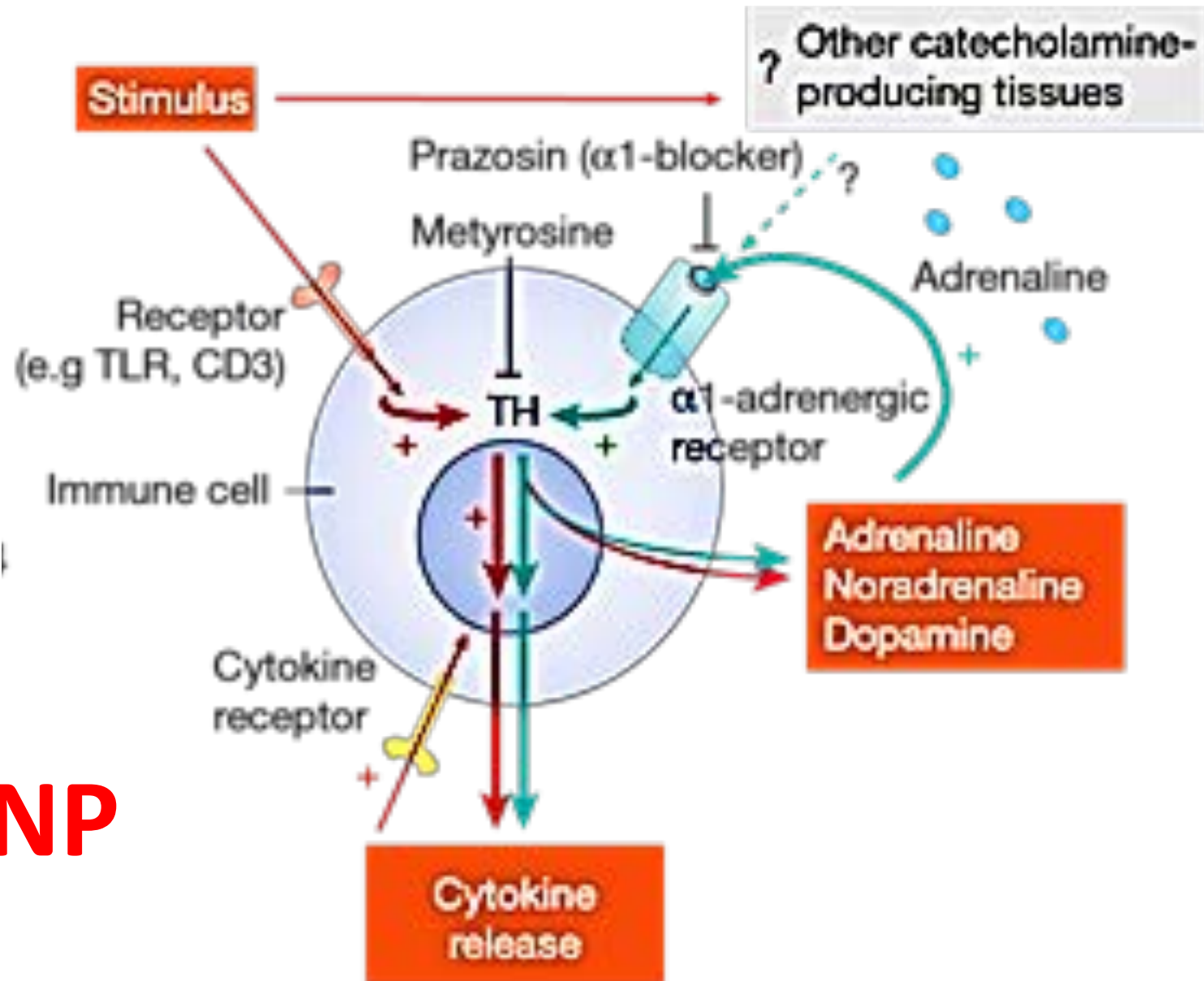
Lesser cytokine increase with MTR

Similar result with ANP

The need for a better model



Conclusions



ANP

This is
my
thank you
dance!

