BACTERIAL TRANSLOCATION AFTER HEMORRHAGIC STROKE. EFFECT OF THE HEMATOMA SIZE AND THE ABSENCE OF TLR4.

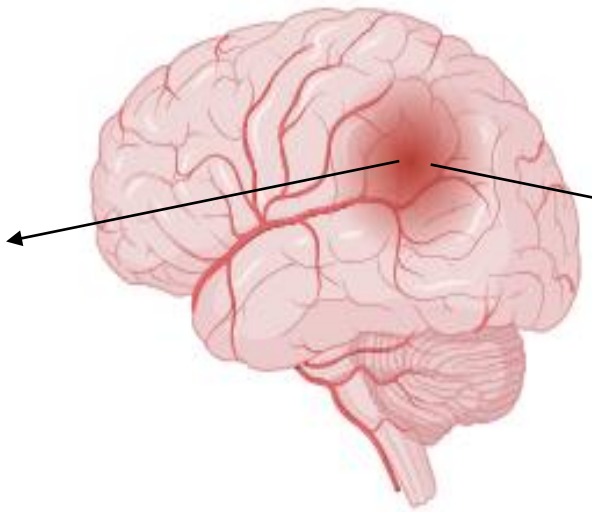
NURIA ALFAGEME
Thesis directors: IGNACIO LIZASOAIN
JESÚS PRADILLO
ENG LO

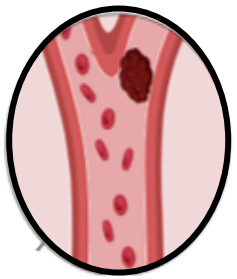
Ischemic Stroke

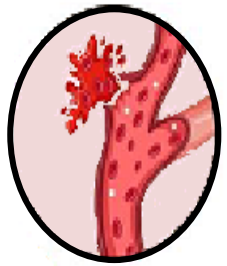
87% of cases.
Leading cause of disability and cognitive deficits.

Second leading cause of death worldwide

Insufficient treatment:
Thrombectomy and tPA.







Hemorrhagic Stroke

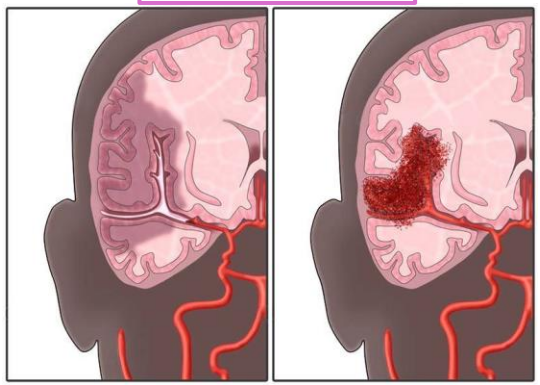
13% of cases.
Higher mortality

No treatment

Only Hospital care

Heart disease and stroke statistics, 2020

STROKE



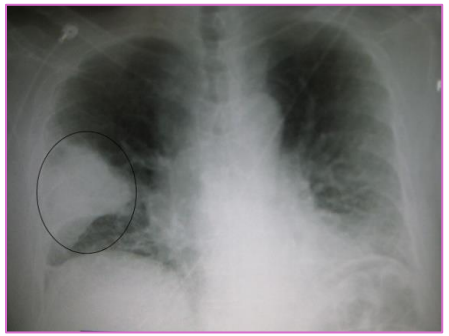
Inflammation

Inmunosupression (IDS)

Infections (Pneumonia & UTIs)

(Westendorp, W.F., et al., BMC Neurol, 2011)

GUT BACTERIAL TRANSLOCATION



- **IDS**
- **Dysphagia**
- **Catheters**
- **Immovilization**

nature medicine

Translocation and dissemination of commensal bacteria in post-stroke infection

Dragana Stanley[1], Linda J Mason[2], Kate E Mackin[3,4], Yogitha N Srikhanta[3,4], Dena Lyras[3,4], Monica D Prakash[5], Kulmira Nurgali[5], Andres Venegas[6], Michael D Hill[6], Robert J Moore[3,4,7] & Connie H Y Wong[8]

GUT MICROBES
2017, VOL. 8, NO. 6, 601–606
https://doi.org/10.1080/19490976.2017.1344809

Taylor & Francis
Taylor & Francis Group

ADDENDUM

OPEN ACCESS Check for updates

An unexplored brain-gut microbiota axis in stroke

Shu Wen Wen and Connie H. Y. Wong

Centre for Inflammatory Diseases, Department of Medicine, School of Clinical Sciences, Monash University, Clayton, Victoria, Australia

Received: 6 February 2019 | Revised: 16 April 2019 | Accepted: 12 May 2019
DOI: 10.1111/acel.12980

ORIGINAL ARTICLE

Aging Cell WILEY

Advanced age promotes colonic dysfunction and gut-derived lung infection after stroke

Shu Wen Wen[1] | Raymond Shim[1] | Luke Ho[1,2] | Brooke J. Wanrooy[1] | Yogitha N. Srikhanta[3] | Kathryn Prame Kumar[1] | Alyce J. Nicholls[1] | SJ. Shen[1] | Tara Sepehrizadeh[4] | Michael de Veer[4] | Velandai K. Srikanth[2] | Henry Ma[5] | Thanh G. Phan[5] | Dena Lyras[3] | Connie H. Y. Wong[1]

GUT BACTERIAL TRANSLOCATION

frontiers in Microbiology

Gut Microbiota Dysbiosis Induced by Intracerebral Hemorrhage Aggravates Neuroinflammation in Mice

Xiaobo Yu[1†], Guoyang Zhou[1†], Bo Shao[1,2], Hang Zhou[1], Chaoran Xu[1], Feng Yan[1], Lin Wang[1], Gao Chen[1], Jianru Li[1] and Xiongjie Fu[1*]*

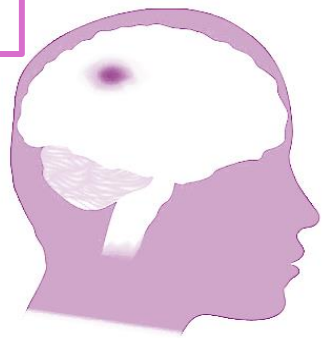
GUT BACTERIAL TRANSLOCATION

frontiers in Immunology

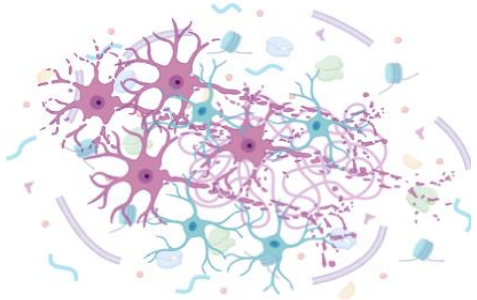
Dynamic Process of Secondary Pulmonary Infection in Mice With Intracerebral Hemorrhage

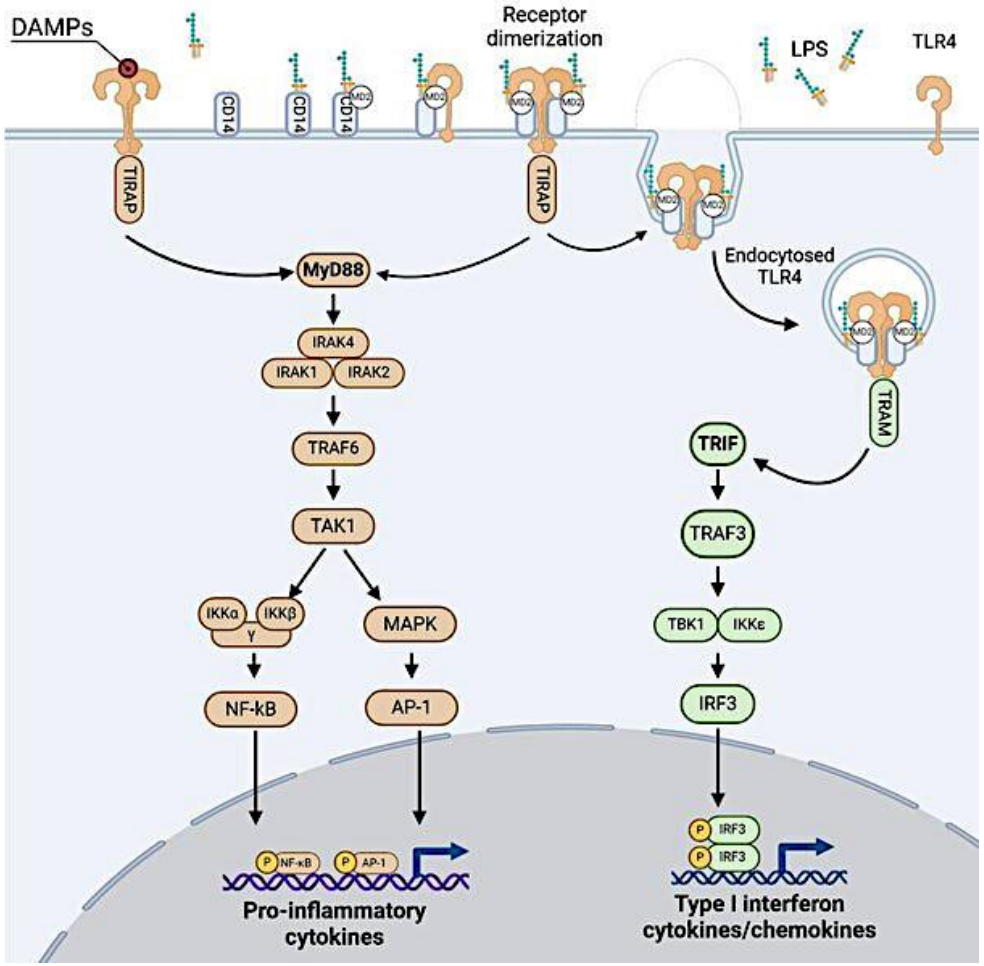
Hanyu Zhang[1,2†], Yingying Huang[1,2,3†], Xiaojin Li[1,2], Xu Han[1,2], Jing Hu[1,2], Bin Wang[1,2], Lin Zhang[1,2], Pengwei Zhuang[1,2] and Yanjun Zhang[1,2*]*

TLR4 RECEPTOR



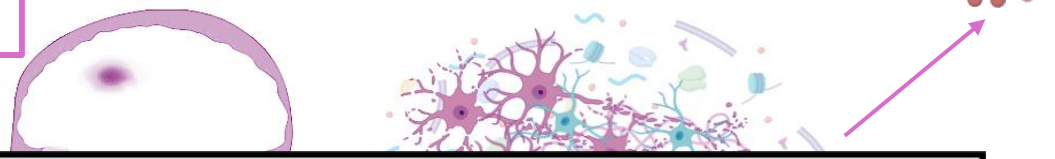
Damage Brain Tissue





TLR4 RECEPTOR

Demage Brain Tissue



Circulation

JOURNAL OF THE AMERICAN HEART ASSOCIATION

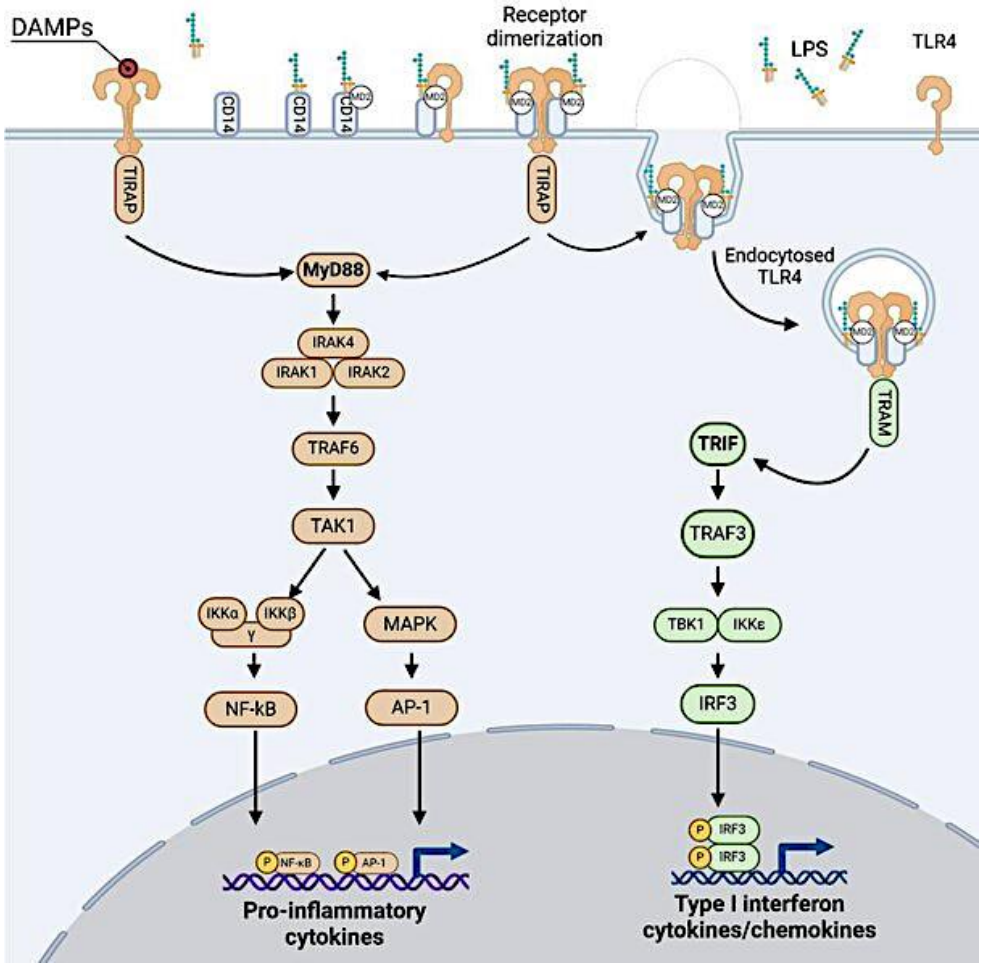
American Heart Association. Learn and Live.

Toll-Like Receptor 4 Is Involved in Brain Damage and Inflammation After Experimental Stroke

Javier R. Caso, Jesús M. Pradillo, Olivia Hurtado, Pedro Lorenzo, María A. Moro and Ignacio Lizasoain

Circulation published online Mar 19, 2007;
DOI: 10.1161/CIRCULATIONAHA.106.603431
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514

 Print ISSN: 0009-7322. Online ISSN: 1524-4539



TLR4 RECEPTOR

Demage Brain Tissue

Circulation
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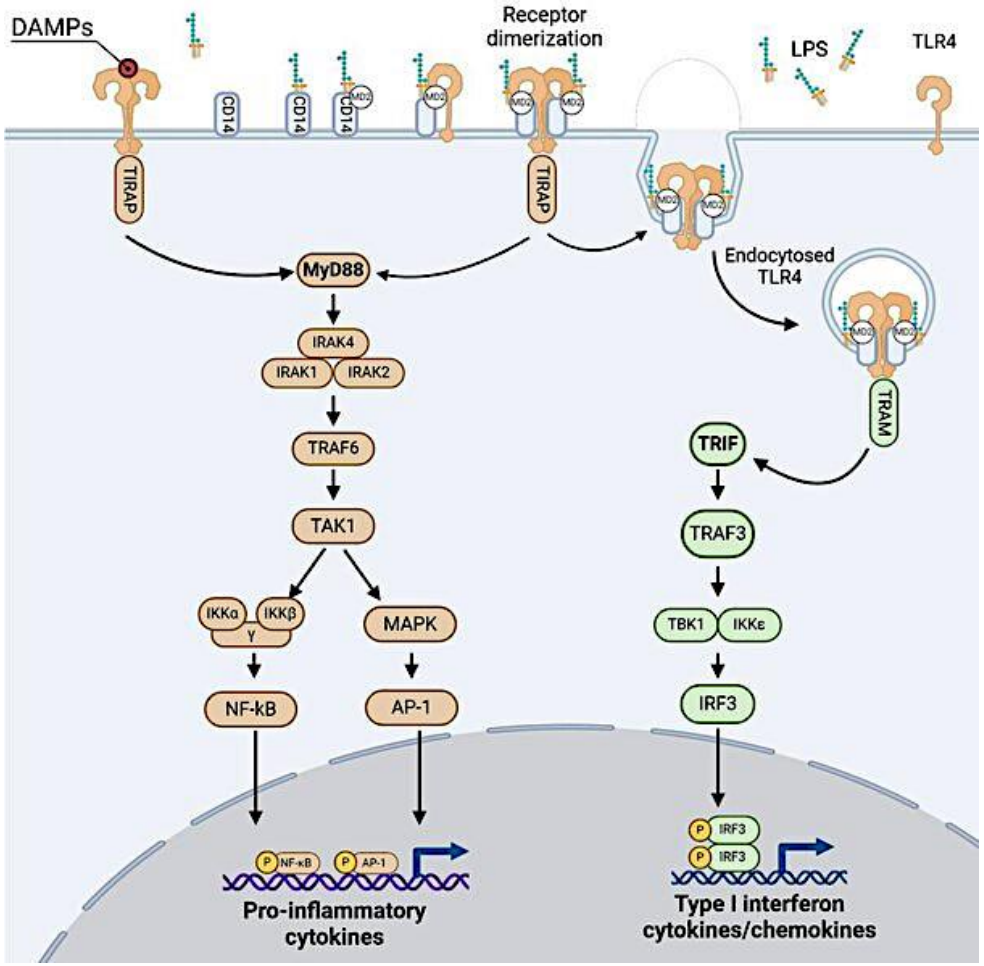
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

Stroke

Brief Report

Toll-Like Receptor 4 Mediates Hemorrhagic Transformation After Delayed Tissue Plasminogen Activator Administration in In Situ Thromboembolic Stroke

Alicia García-Culebras, MSc*; Sara Palma-Tortosa, MSc*; Ana Moraga, PhD; Isaac García-Yébenes, PhD; Violeta Durán-Laforet, MSc; Maria I. Cuartero, PhD; Juan de la Parra, MSc; Ana L. Barrios-Muñoz, MSc; Jaime Díaz-Guzmán, MD, PhD; Jesús M. Pradillo, PhD; María A. Moro, PhD†; Ignacio Lizasoain, MD, PhD†



JOURNAL OF NEUROINFLAMMATION

RESEARCH Open Access

Heme activates TLR4-mediated inflammatory injury via MyD88/TRIF signaling pathway in intracerebral hemorrhage

Sen Lin[1,4†], Qing Yin[2†], Qi Zhong[1], Feng-Lin Lv[3], Yu Zhou[1], Jing-Qi Li[1], Jing-Zhou Wang[1], Bing-yin Su[4*] and Qing-Wu Yang[1*]

Stroke

Volume 44, Issue 9, September 2013; Pages 2545-2552

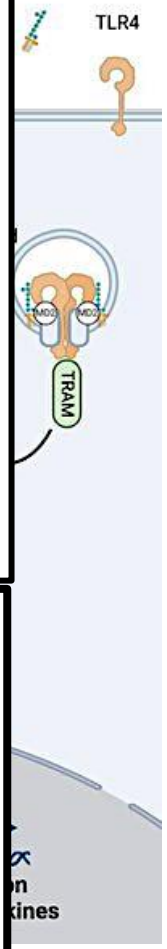
https://doi.org/10.1161/STROKEAHA.113.001038

American Heart Association.

BASIC SCIENCES

Toll-Like Receptor 4 Antagonist Attenuates Intracerebral Hemorrhage–Induced Brain Injury

Yan-Chun Wang, BS, Peng-Fei Wang, MS, Huang Fang, MS, Jing Chen, BS, Xiao-Yi Xiong, BS, and Qing-Wu Yang, PhD, MD

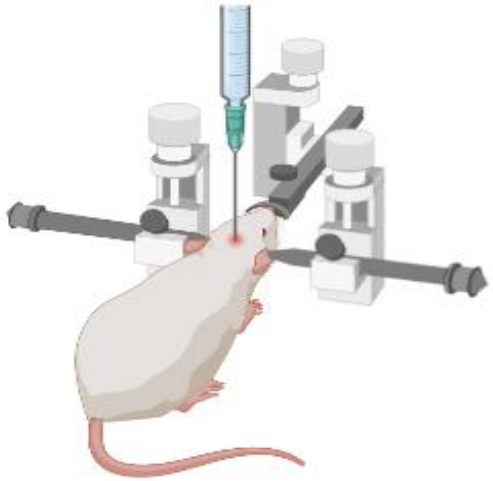


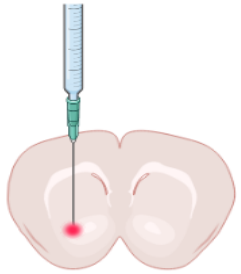
Hypothesis 1: Hemorrhagic stroke (HS) and the infarct volume favors the damage of the gut barrier allowing the process of bacterial translocation (BT).

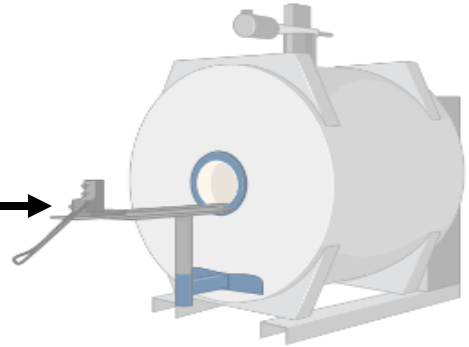
Hypothesis 2: The lack of TLR4 reduces the infarct volume and the process of BT after experimental HS.

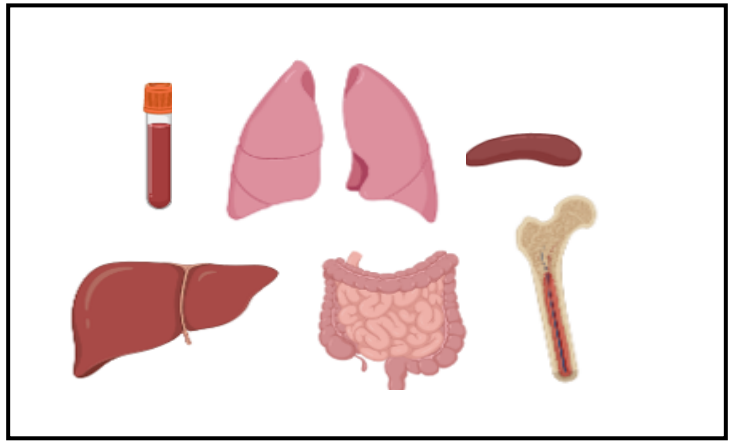
T_2W

72H



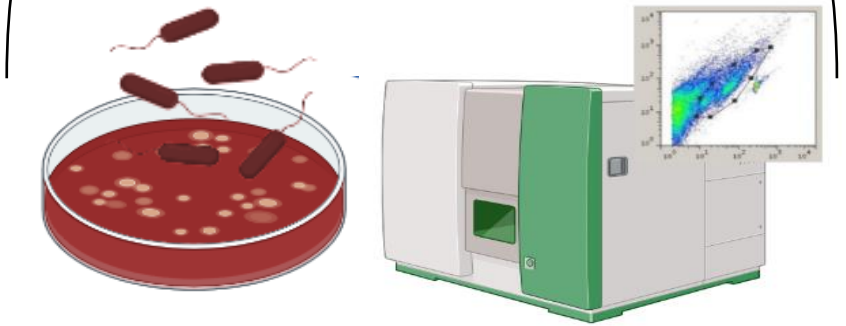






♂ Wistar Rats, 10-12weeks, 300-350g.

♂ C57 Mice (Wild Type and TLR4-KO), 10-12 weeks, 25g



RAT

Naïve: Control group without surgery

Sham: Control group with surgery, but without collagenase.

HS-0.3CDU: Experimental group 1. With 0.3CDUs/µL of collagenase. Subdivided into 0.3NBT and 0.3BT.

HS-0.5CDU: Experimental group 2. With 0.5CDUs/µL of collagenase

MICE

Naïve WT: Control group without surgery Wild Type mice.

Naïve KO: Control group without surgery **TLR4-KO** mice.

HS-WT: Experimental group of Wild Type mice with 0.03CDUs/µL of collagenase. Subdivided into WT-NBT and WT-BT.

HS-KO: Experimental group of **TLR4-KO** mice with 0.03CDUs/µL of collagenase. Subdivided into KO-NBT and KO-BT.

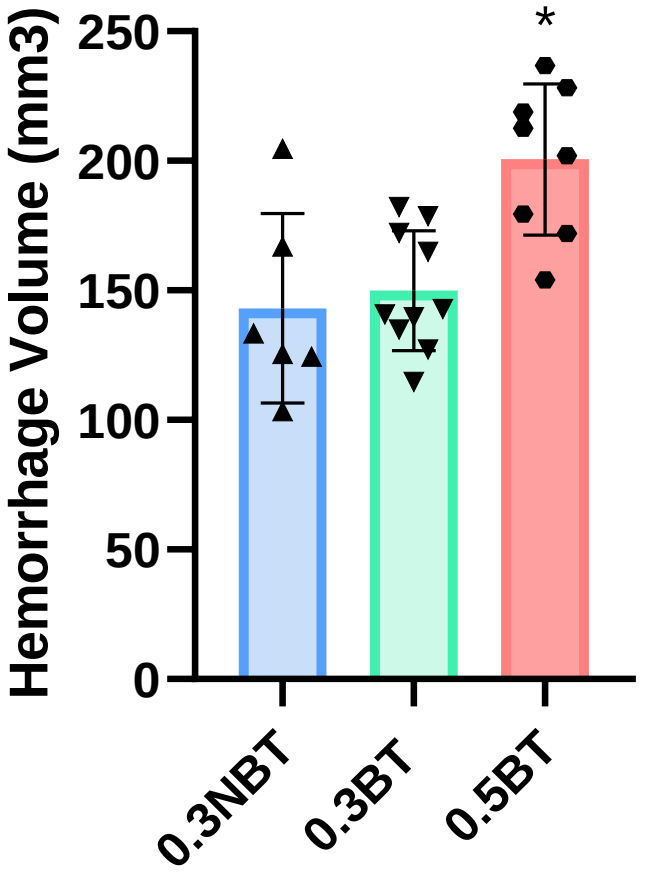
Hypothesis 1: Results in rats

INFARCT VOLUME

0.3 NBT

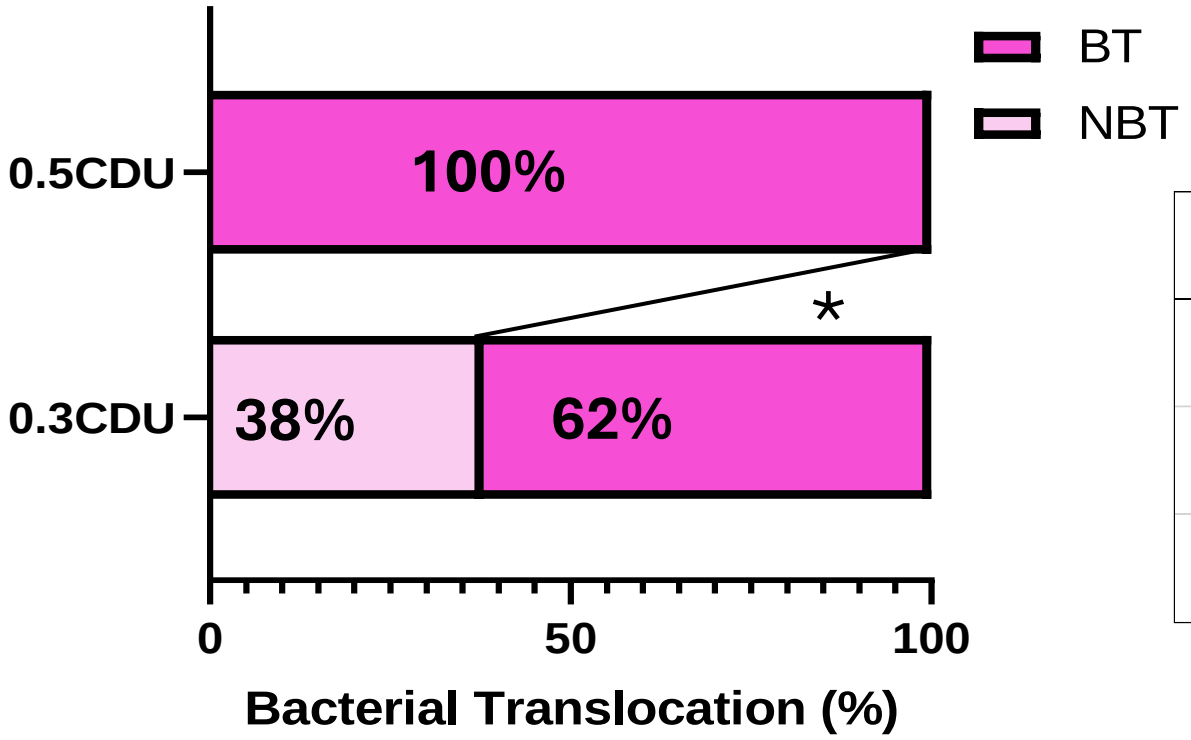
0.3 BT

0.5 BT



Hemorrhage volume (mm3) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 0.5BT vs. 0.3NBT/0.3BT.

BACTERIAL TRANSLOCATION

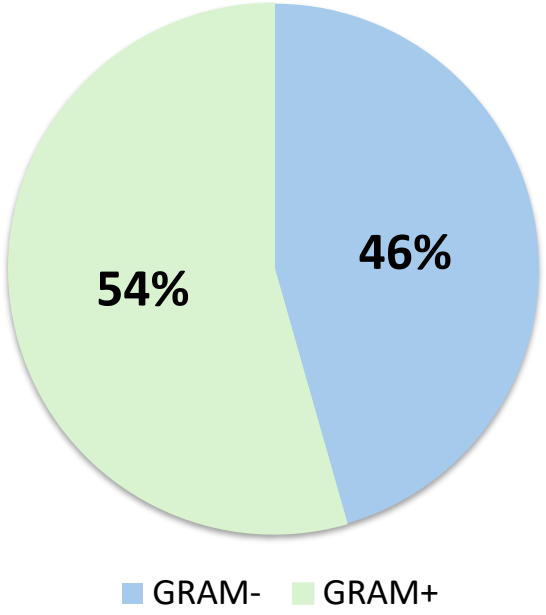


	MN	LIVER	SPLEEN	LUNG
IH-0.3-NTB	0%	0%	0%	0%
IH-0.3-TB	100%	20%	30%	30%
IH- 0.5-TB	88%	50%	13%	25%

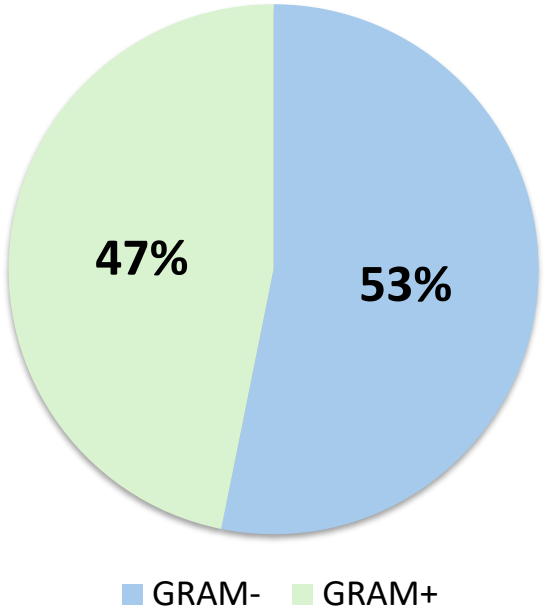
*Percentage of animals in which bacterial colonies were found in the organs analyzed. Chi-square,*p<0.05 0.3 CDUs TB vs. 0.5 CDUs TB.*

MICORBIOLOGICAL ANALYSIS

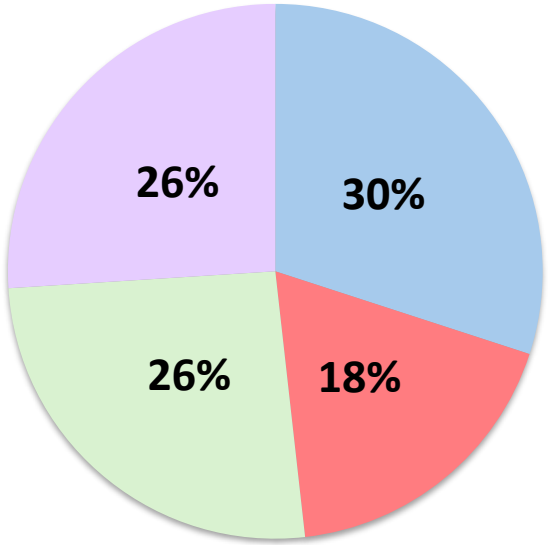
TOTAL GRAM+/GRAM- 0.3CDU



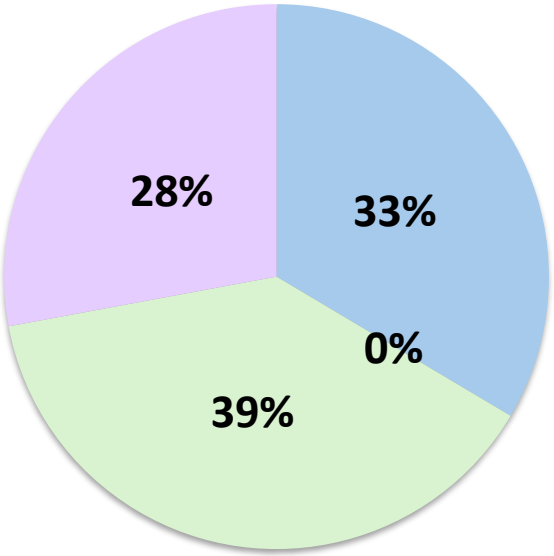
TOTAL GRAM+/GRAM- 0.5CDU



TOTAL BACTERIAL TRANSLOCATION 0.3CDU



TOTAL BACTERIAL TRANSLOCATION 0.5 CDU

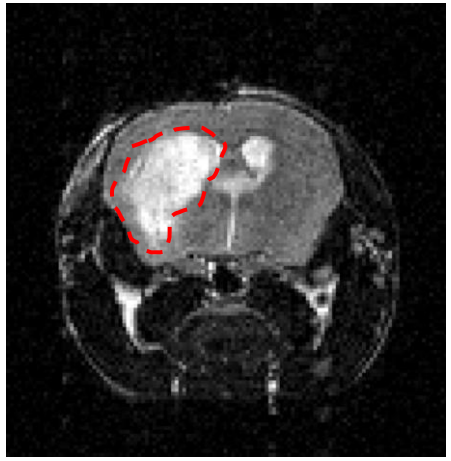


FIRMICUTES	PROTEOBACTERIAS
BACTEROIDETES	ACTINOBACTERIAS

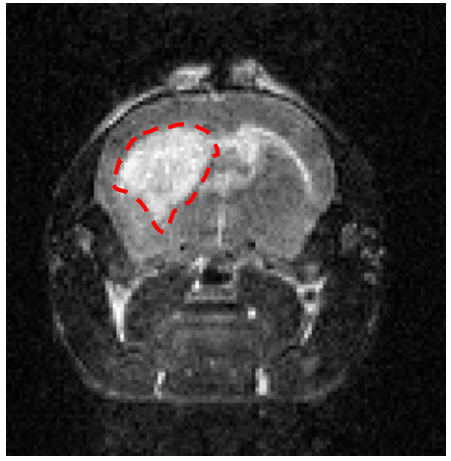
Hypothesis 2: Results in mice

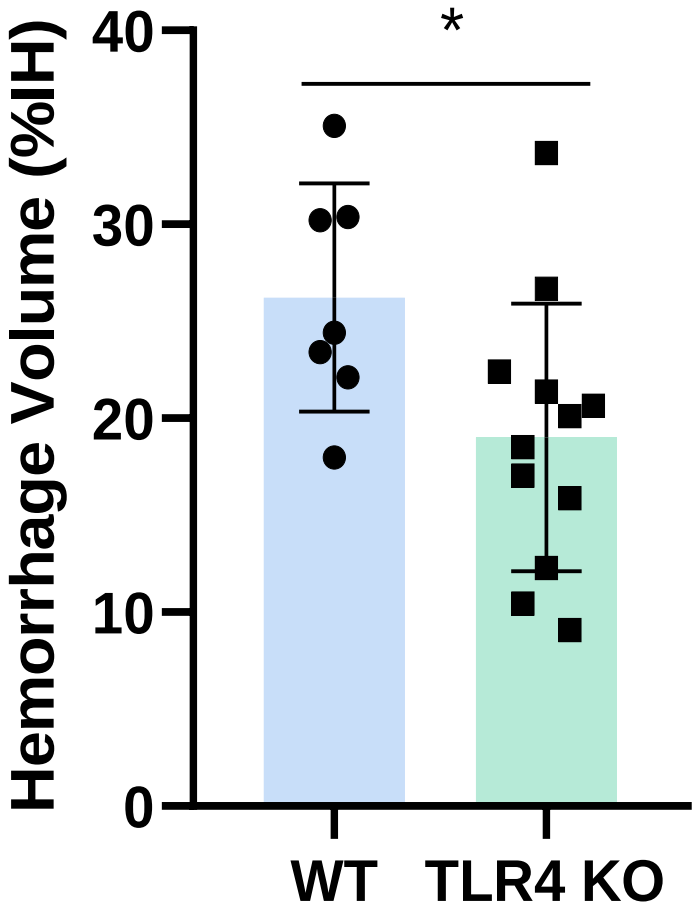
INFARCT VOLUME

WT



TLR4KO

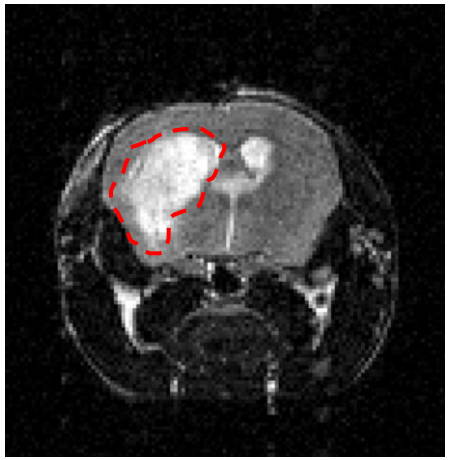




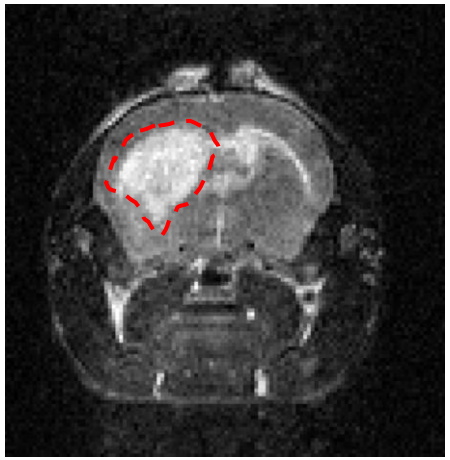
Hemorrhage volume (% IH) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=7-12 per experimental group. *p<0.05 WT vs. TLR4 KO.

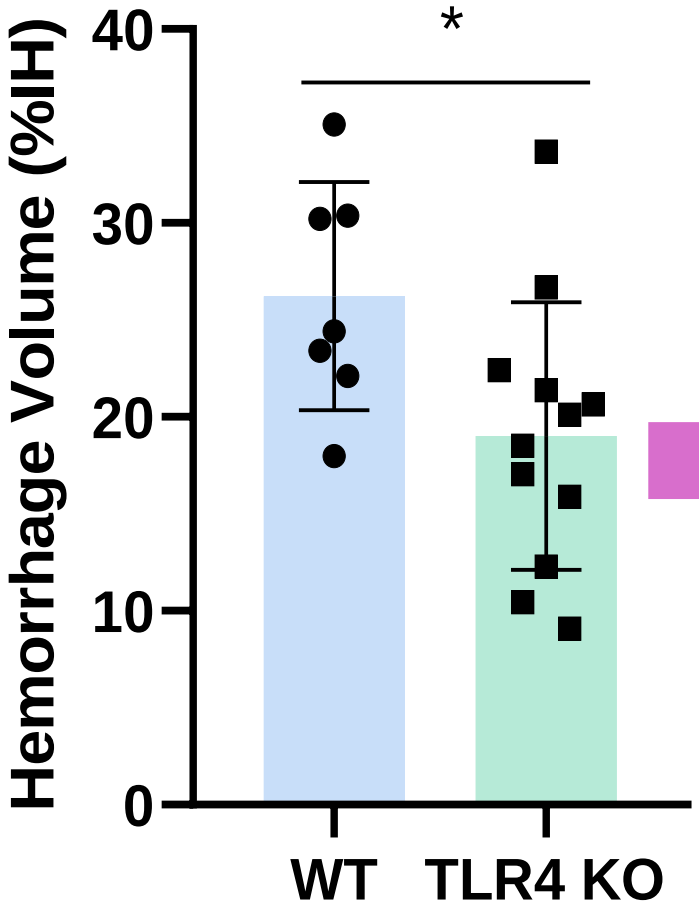
INFARCT VOLUME

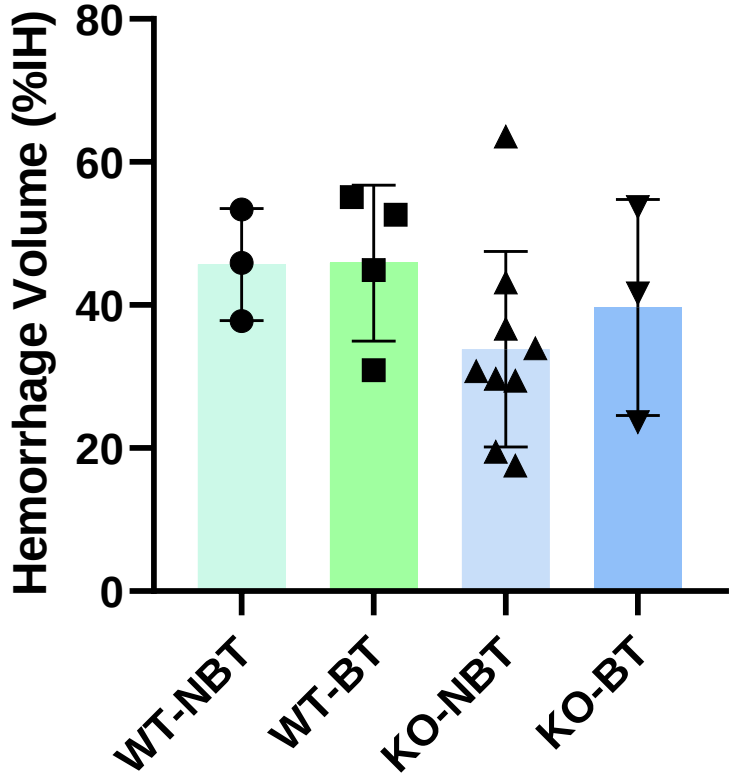
WT



TLR4KO

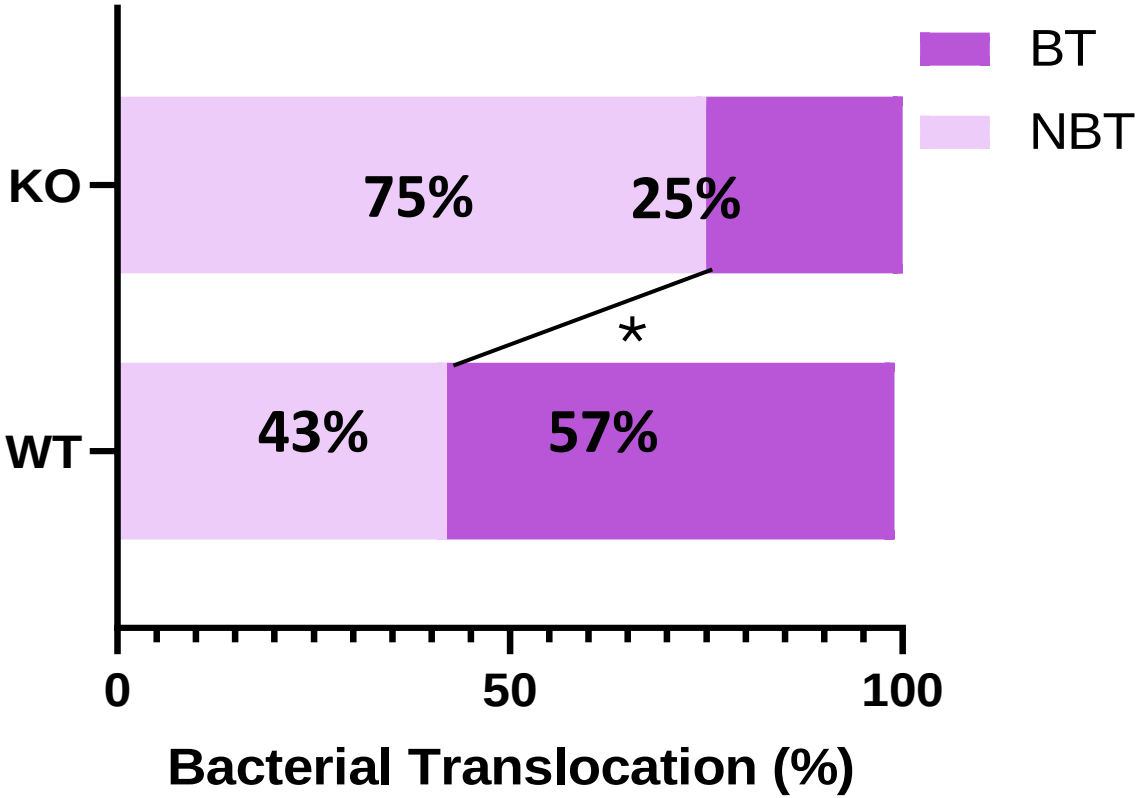






Hemorrhage volume (% IH) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=7-12 per experimental group. *p<0.05 WT vs. TLR4 KO.

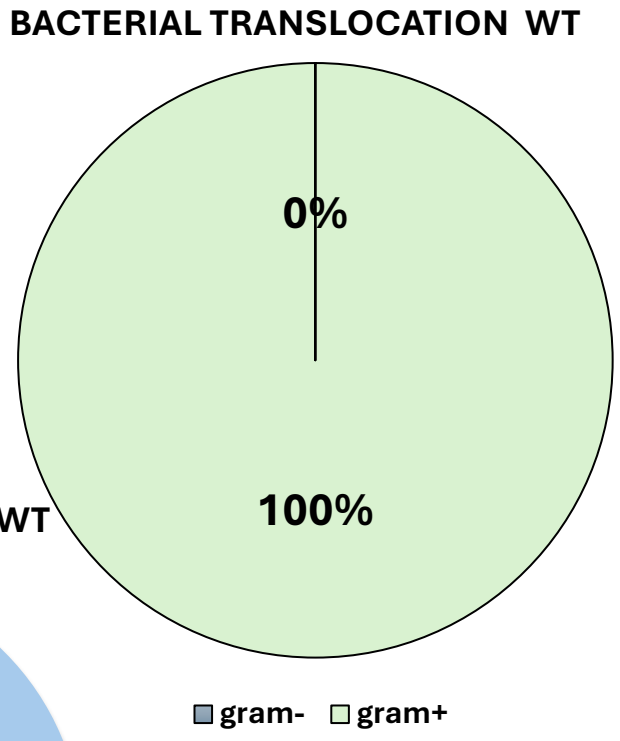
BACTERIAL TRANSLOCATION

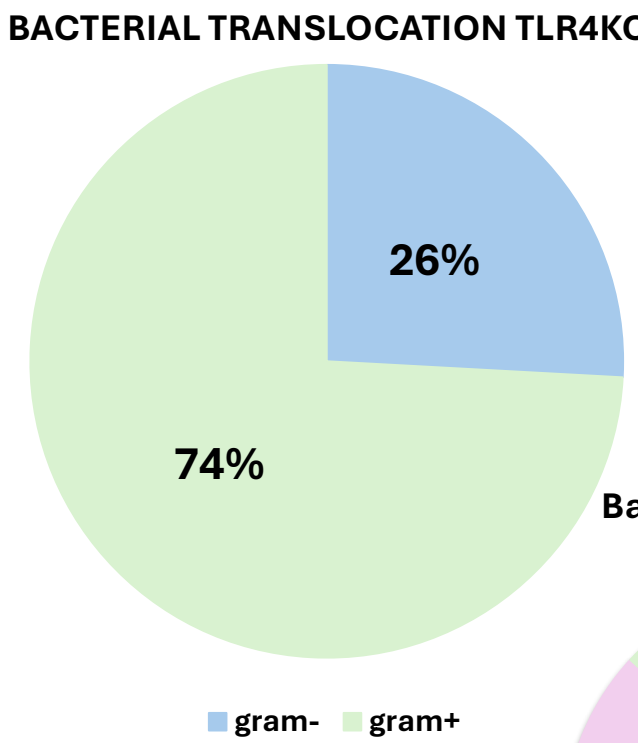


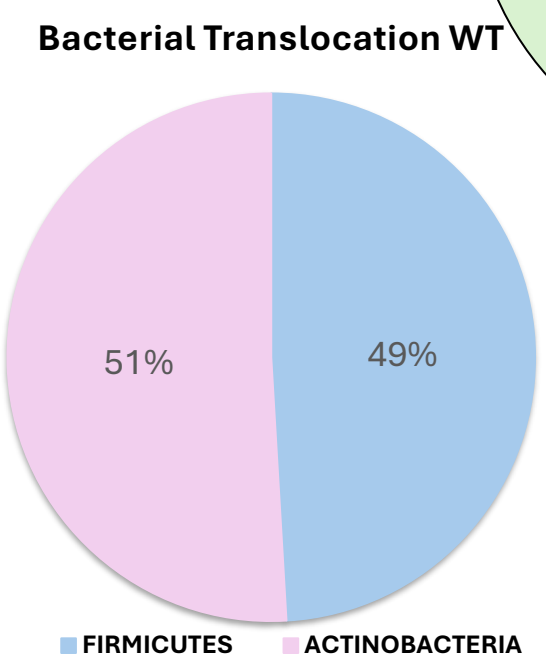
*Percentage of animals in which bacterial colonies were found in the organs analyzed. Chi-square,*p<0.05 WT-BT vs. KO-BT.*

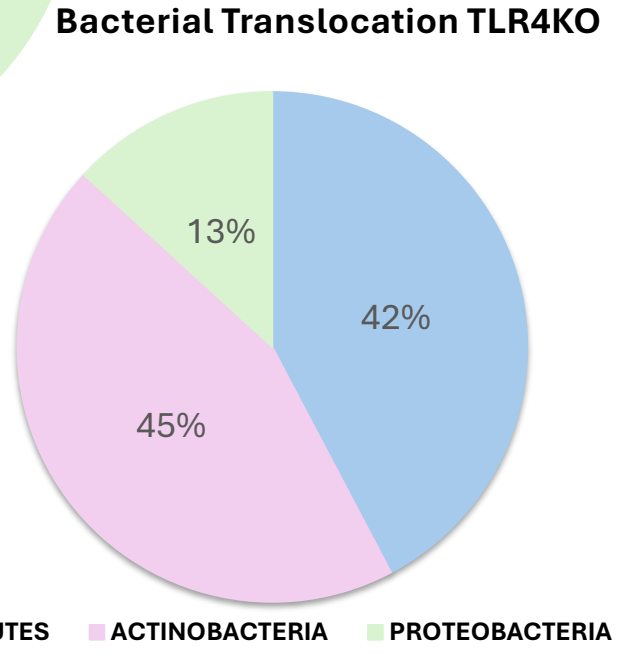
	MN	LIVER	SPLEEN	LUNG
WT-NBT	0%	0%	0%	0%
WT-BT	100%	0%	0%	0%
KO-NBT	0%	0%	0%	0%
KO-TB	100%	33%	33%	33%

MICORBIOLOGICAL ANALYSIS



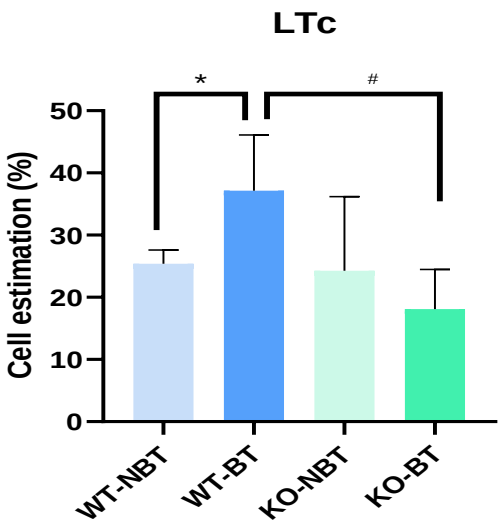




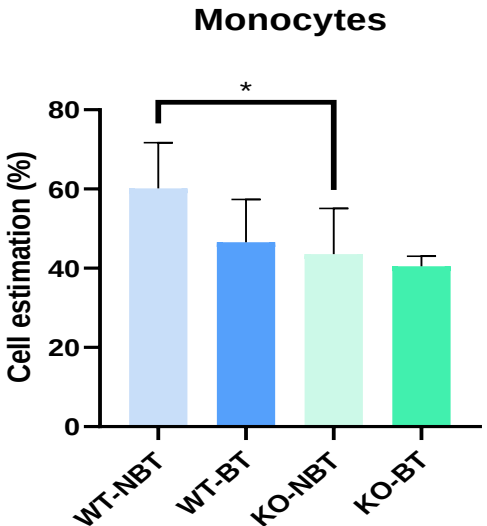


PERIPHERAL INFLAMMATION

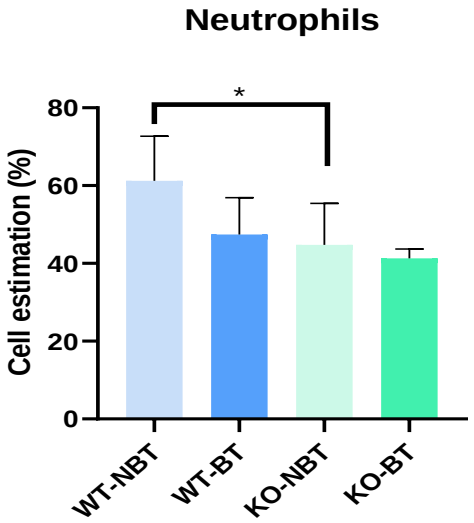
BM



Cell estimation(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p=0.05 WT-NBT vs WT-BT. #p=0.05 WT-BT vs. KO-BT

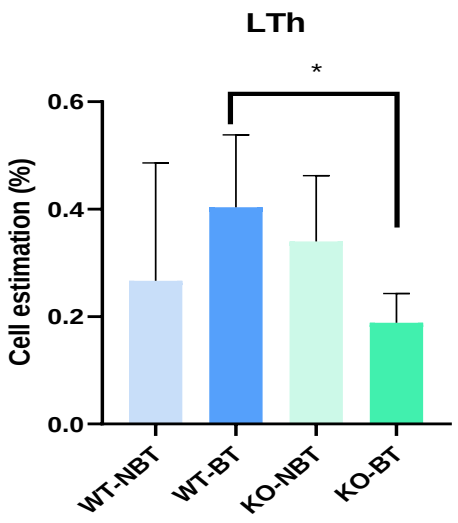


Cell estimation(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. p*=0.05 WT-NBT vs. KO-NBT

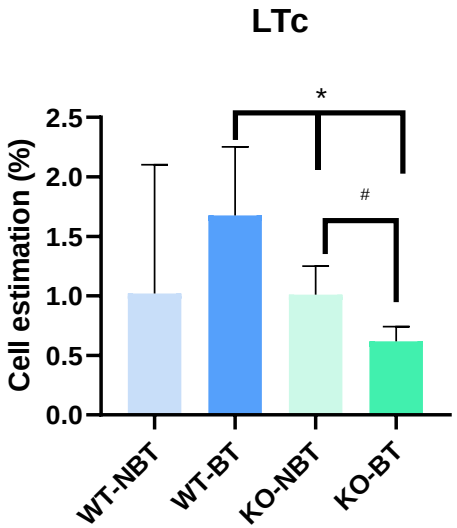


Cell estimation(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. p*=0.05 WT-NBT vs. KO-NBT

Blood



Cell estimation(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. p*=0.05



Cell estimation(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. p*=0.05 WT-BT vs. KO-NBT, KO-BT. p#<0.05 KO-NBT vs KO-BT

Hypothesis 1

1. Bacterial translocation seems to be dependent on the volume of hemorrhage.
2. The diversity of bacterial families found outside the gut seems to be reduced in animals with larger hemorrhage (0.5 BT-group).

Hypothesis 2

3. The absence of the TLR4 reduces the volume of hemorrhage and reduces BT.
4. The lack of TLR4 seems to change the bacterial diversity that translocate.
5. The absence of TLR4 reduces the immune alterations observed in BM and blood compared specifically to WT with BT.

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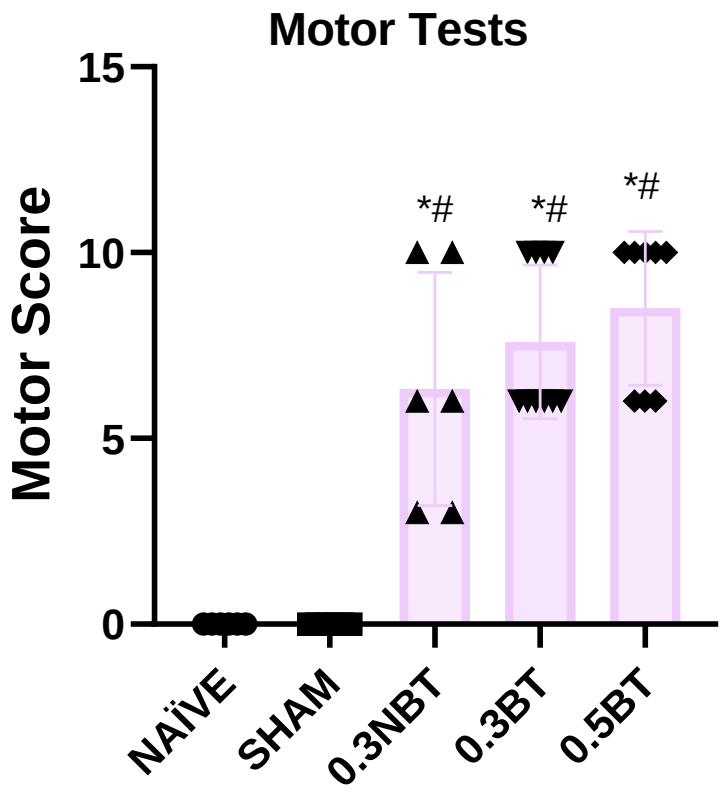
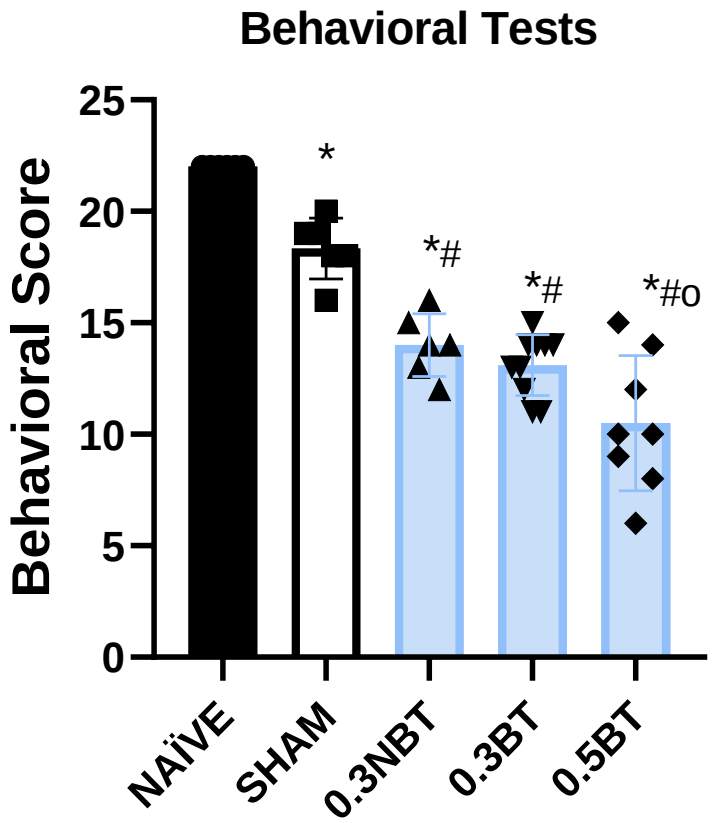
Instituto de Salud Carlos III

cnic



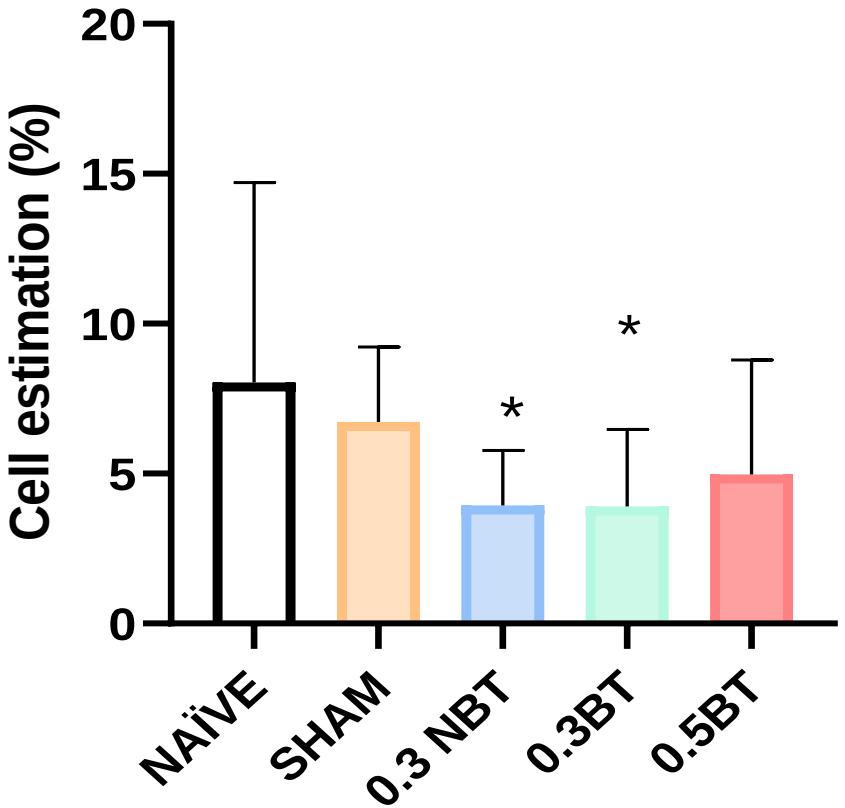
Motor Score at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 NAÏVE vs. 0.3NBT/0.3BT/0.5BT; #p<0.05 SHAM vs. 0.3NBT/0.3BT/0.5BT. Mann-Whitney test.



Behavioral Score at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p <0.05 NAÏVE vs. SHAM/0.3NBT/0.3BT/0.5BT/0.5BT; #p <0.05 SHAM vs. 0.3NBT/0.3BT/0.5BT; op <0.05 0.3NBT vs. 0.5BT. Mann-Whitney test.Trend p=0.0613 0.3BT vs. 0.5BT.

BM

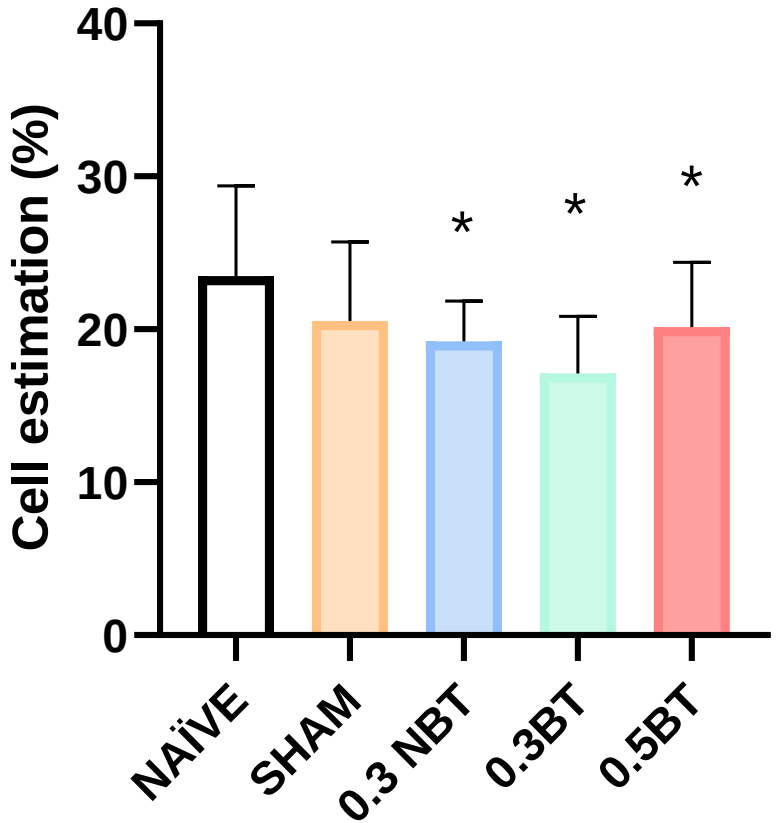
LB



Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 NAÏVE vs. 0.3NBT/0.3BT/0.5BT

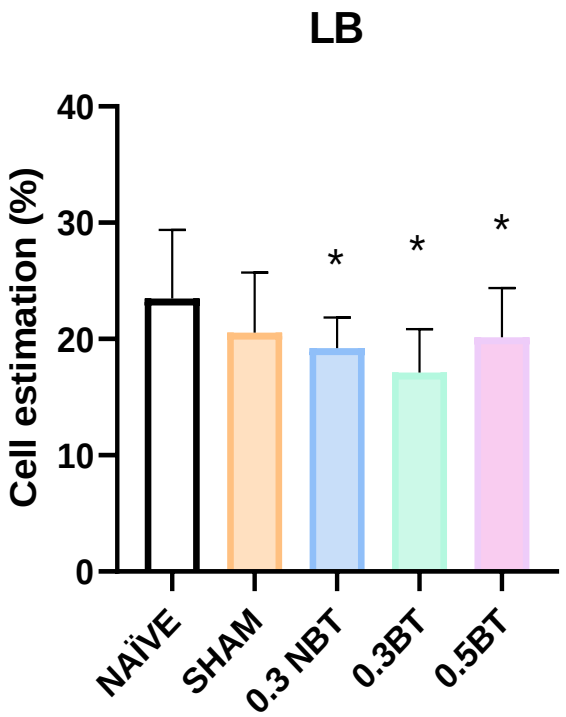
Blood

LB



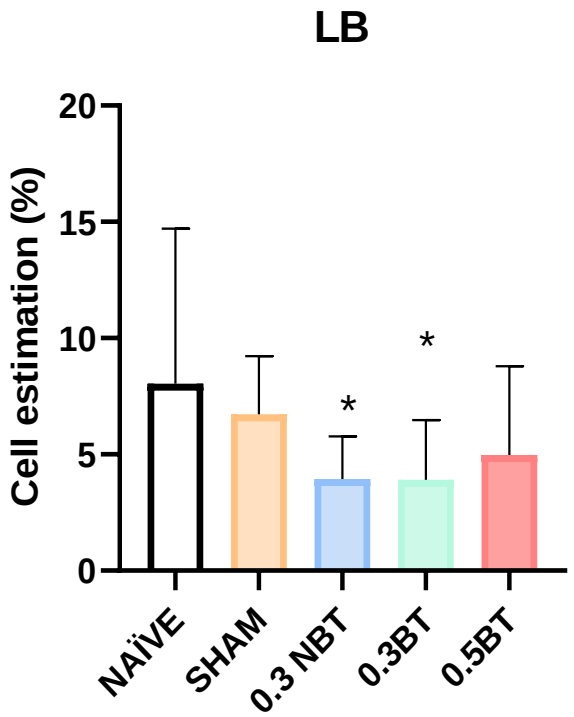
Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. #P<0.05 SHAM vs. 0.3NBT/0.5BT;. Mann-Whitney test.

BM



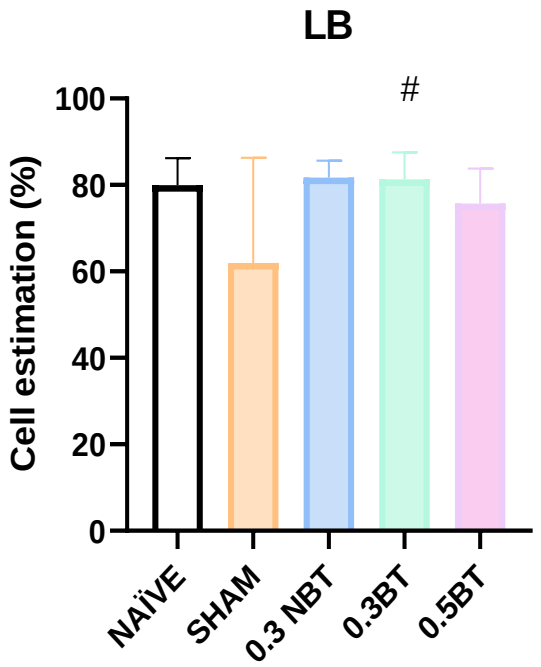
Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 NAÏVE vs. 0.3NBT/0.3BT/0.5BT

Blood

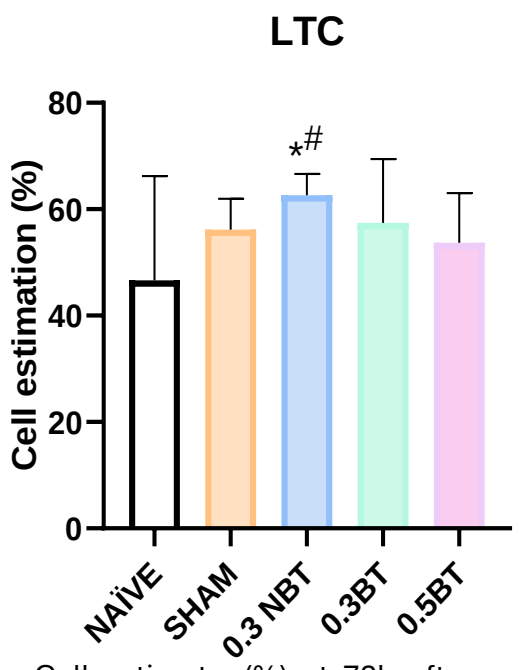


Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. #P<0.05 SHAM vs. 0.3NBT/0.5BT;. Mann-Whitney test.

Spleen



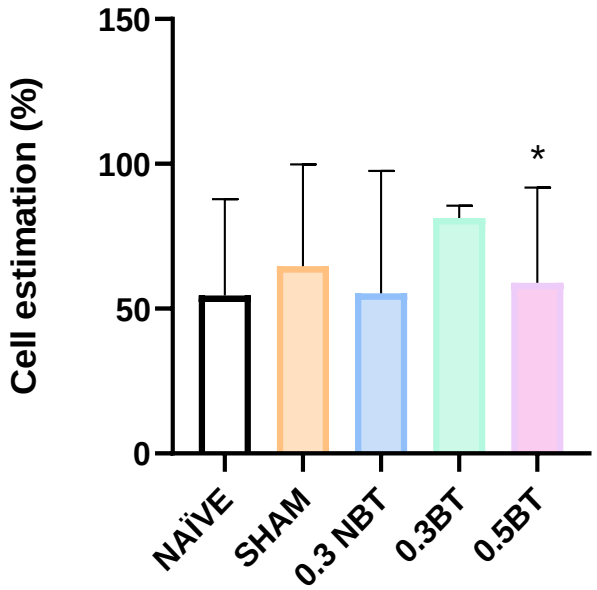
Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. #p<0.05 SHAM vs. 0.3BT. Mann-Whitney test



Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 NAÏVE vs. 0.3NBT/; #p<0.05 SHAM vs. 0.3NBT;

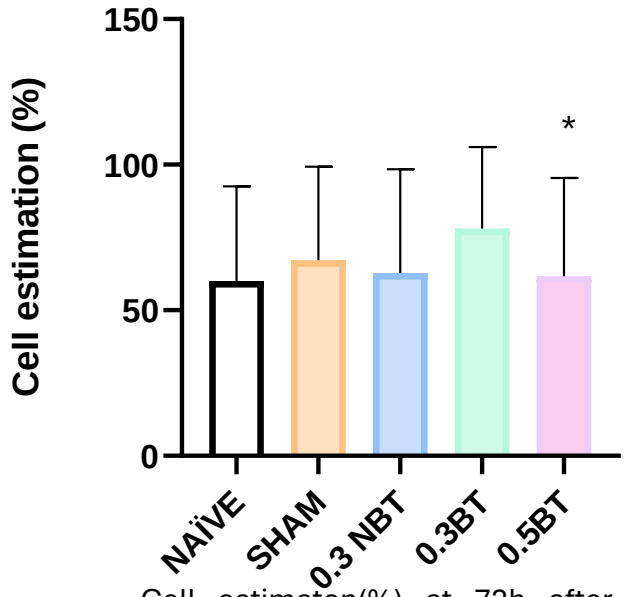
GM

LTC



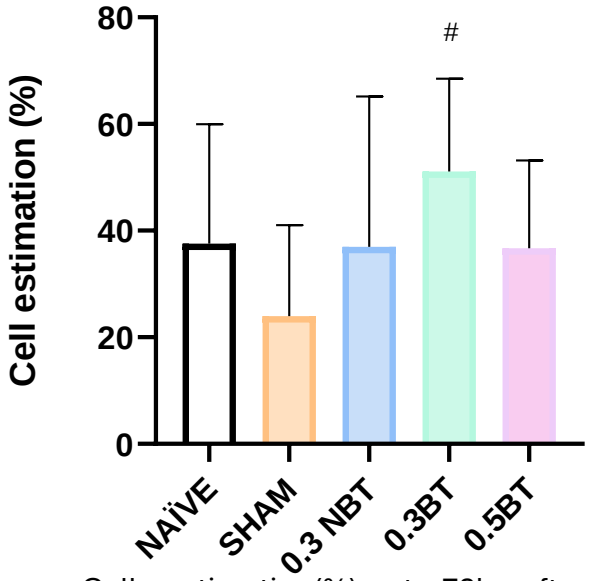
Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p<0.05 0.3BT vs. 0.5BT. Mann-Whitney test.

LTH

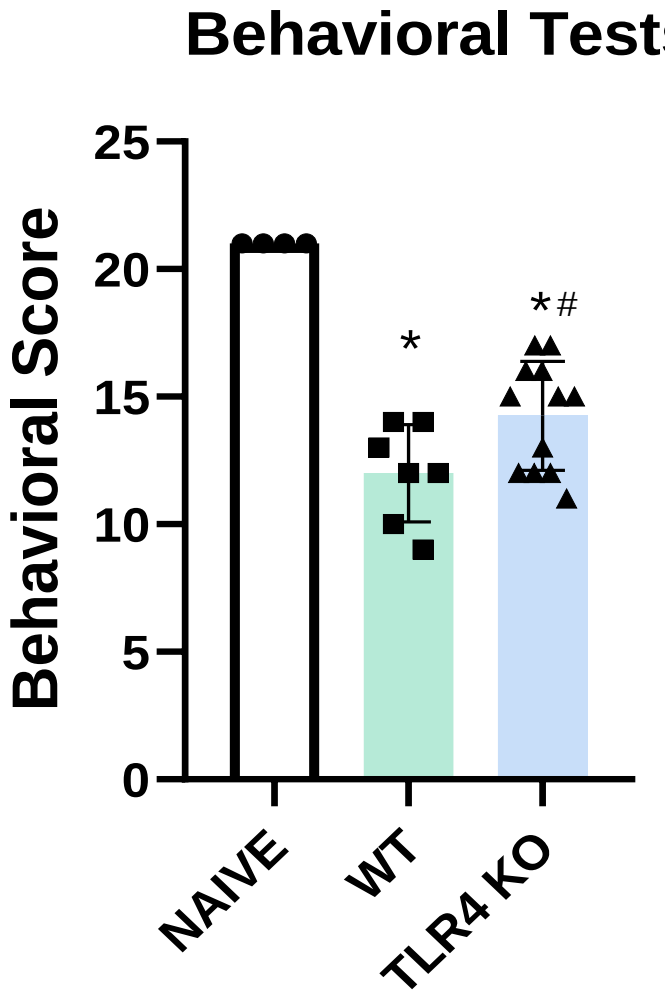


Cell estimaton(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. *p <0.05 0.5BT vs. 0.3BT. Mann-Whitney test

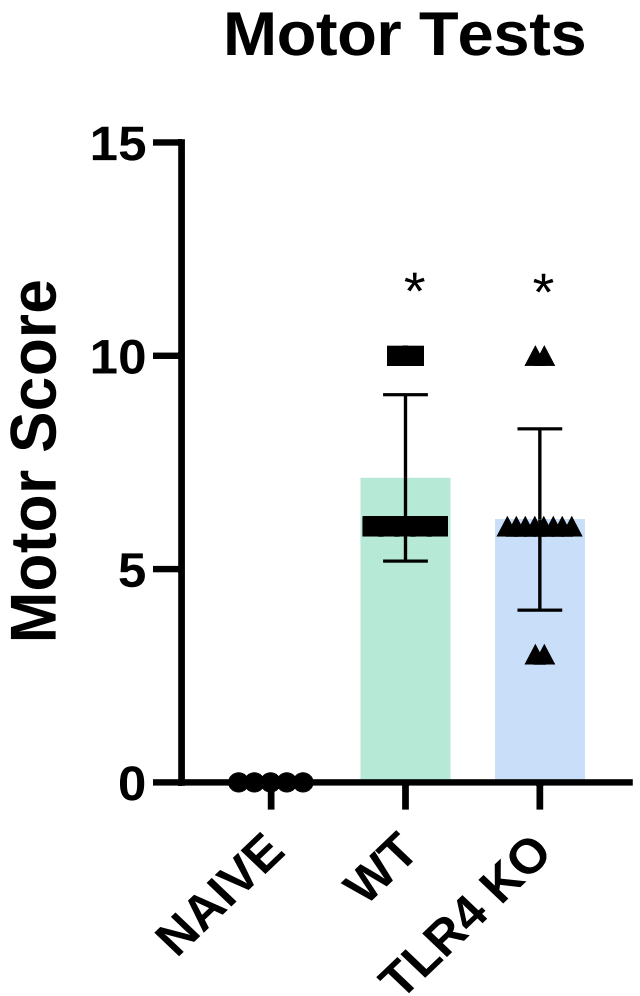
MONOCYTES



Cell estimation(%) at 72h after infarction with 0.3 CDUs of NBT and BT vs. 0.5 CDUs of BT. n=6-10 per experimental group. #p<0.05 SHAM vs. 0.3BT. Mann-Whitney test.



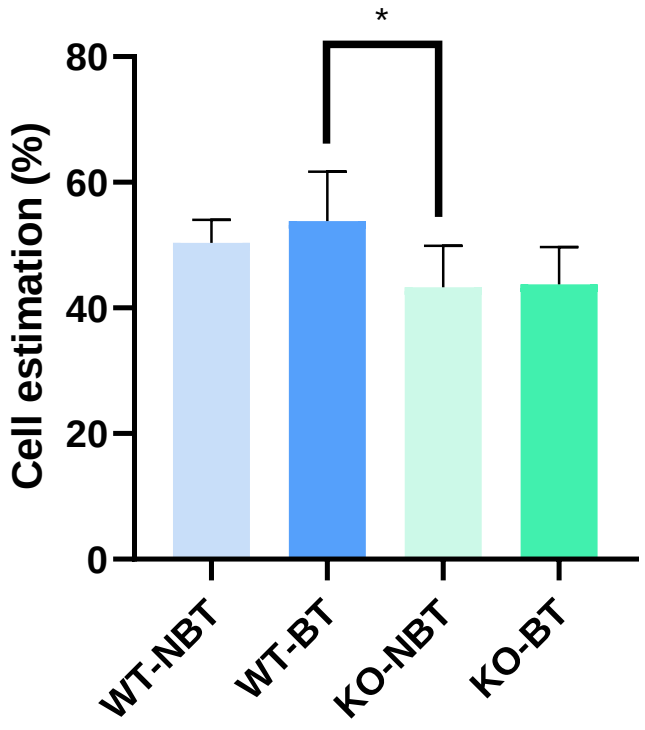
Behavioral Score at 72h after infarction with 0.03 CDUs TLR4KO vs. WT. n=7-12 per experimental group. *p<0.05 NAÏVE vs. TLR4KO/WT; #p=0.05 TLR4KO vs. WT. Mann-Whitney test.



Motor Score at 72h after infarction with 0.03 CDUs TLR4KO vs. WT. n=7-12 per experimental group. *p<0.05 NAÏVE vs. TLR4KO/WT. Mann-Whitney test.

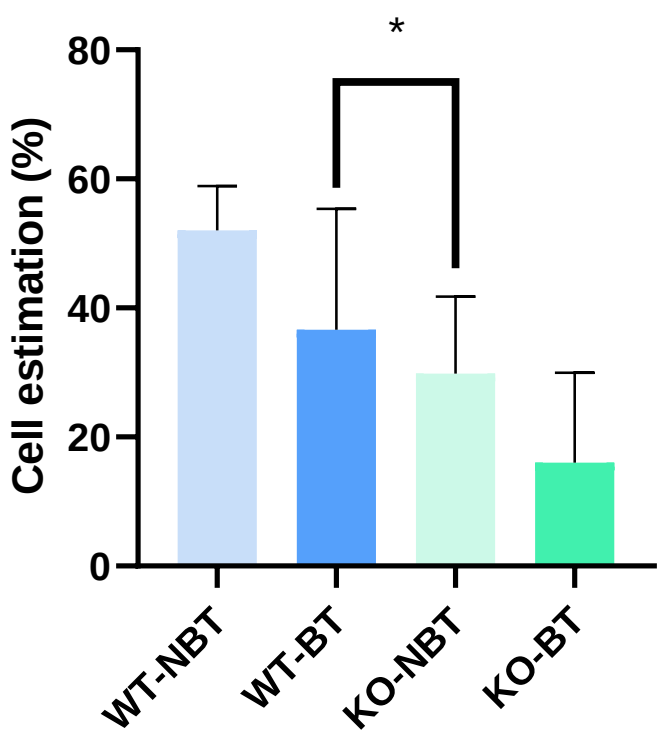
Spleen

LTh



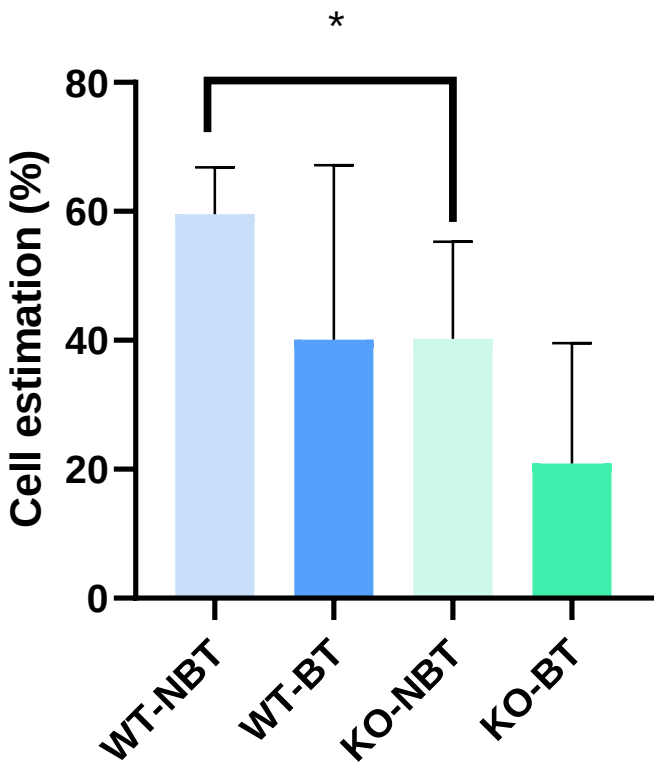
Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. p*<0.05 WT-BT vs KO-NBT

Monocytes



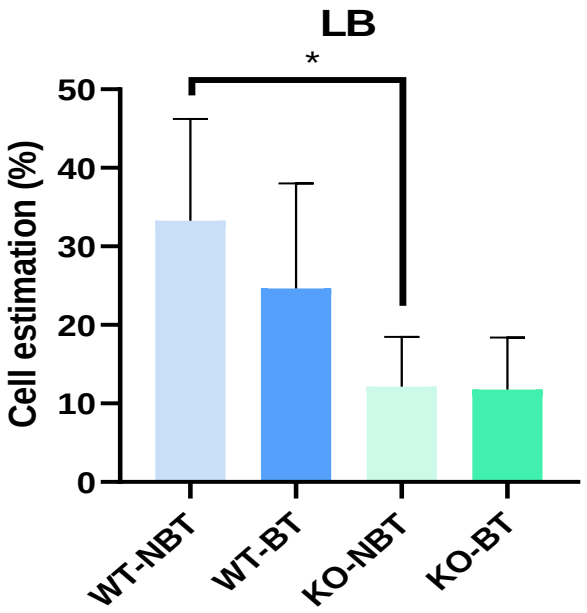
Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p<0.05 WT-NBT vs KO-NBT

Neutrophils

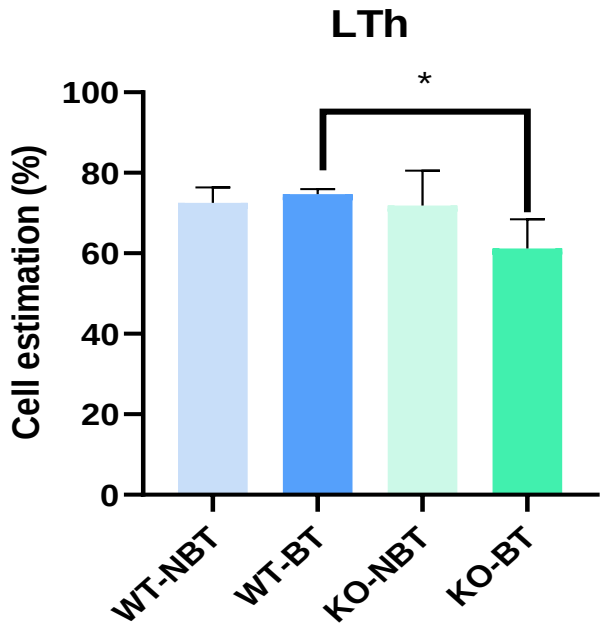


Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group.. *p<0.05 WT-NBT vs KO-NBT

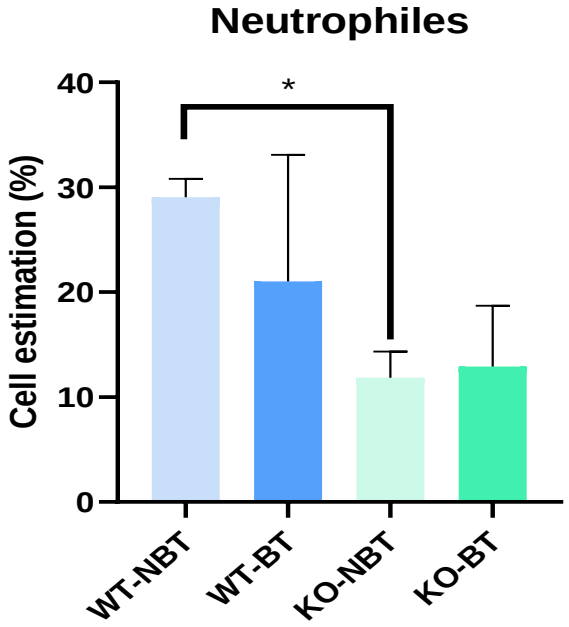
NM



Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p=0.05 WT-NBT vs. KO-NBT.

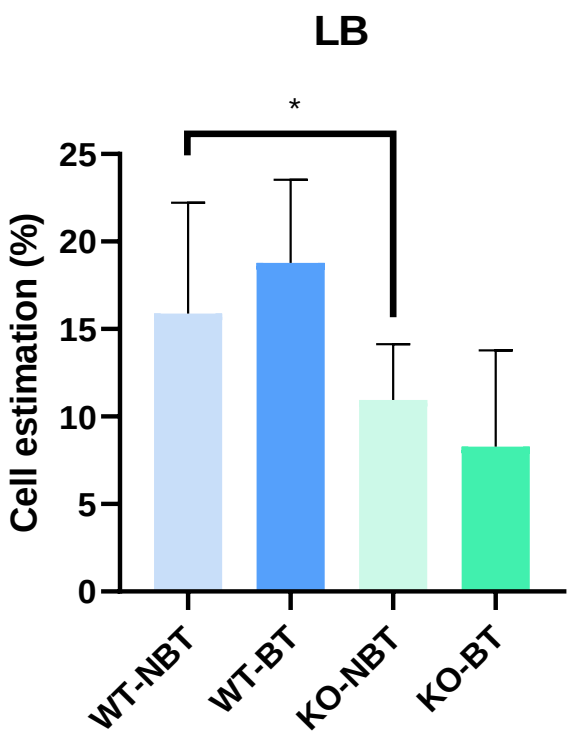


Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p<0.05 WT-BT vs. KO-BT.

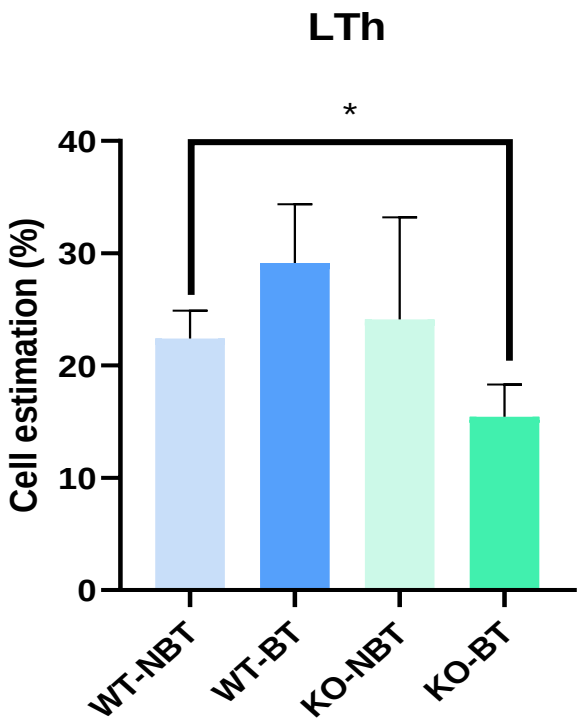


Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p=0.05 WT-NBT vs. KO-NBT.

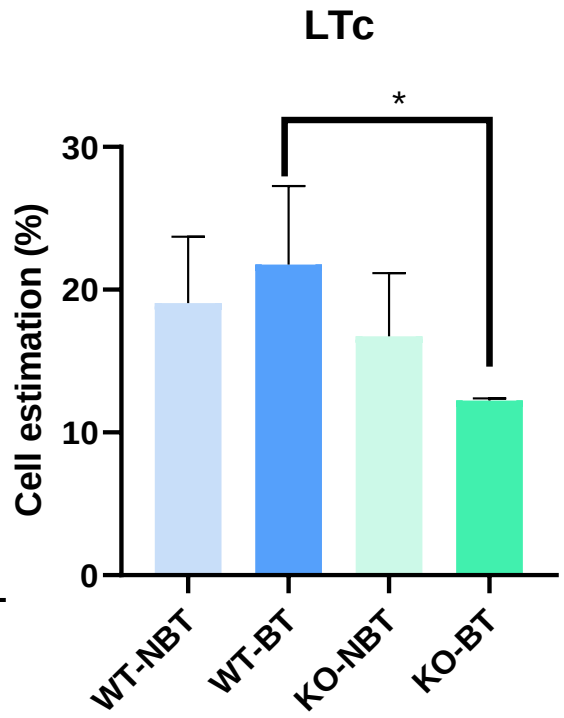
Lung



Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p<0.05 NBH-WT vs. NBH-KO,KO-NBT, KO-BT. #p<0.05 NBH-KO vs. WT-BT. p°=0.05 WT-NBT vs. KO-NBT.



Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p=0.05 WT-NBT vs. KO-BT.



Cell estimaton(%) at 72h after stroke with 0.03 CDUs. n=7-12 per experimental group. *p<0.05 WT-BT vs. KO-BT.