



A Synthetic Gene Regulatory Device for Autonomous Sensing and Suppression of Inflammation

Anže Smole¹, Duško Lainšček¹, Urban Bezeljak¹,

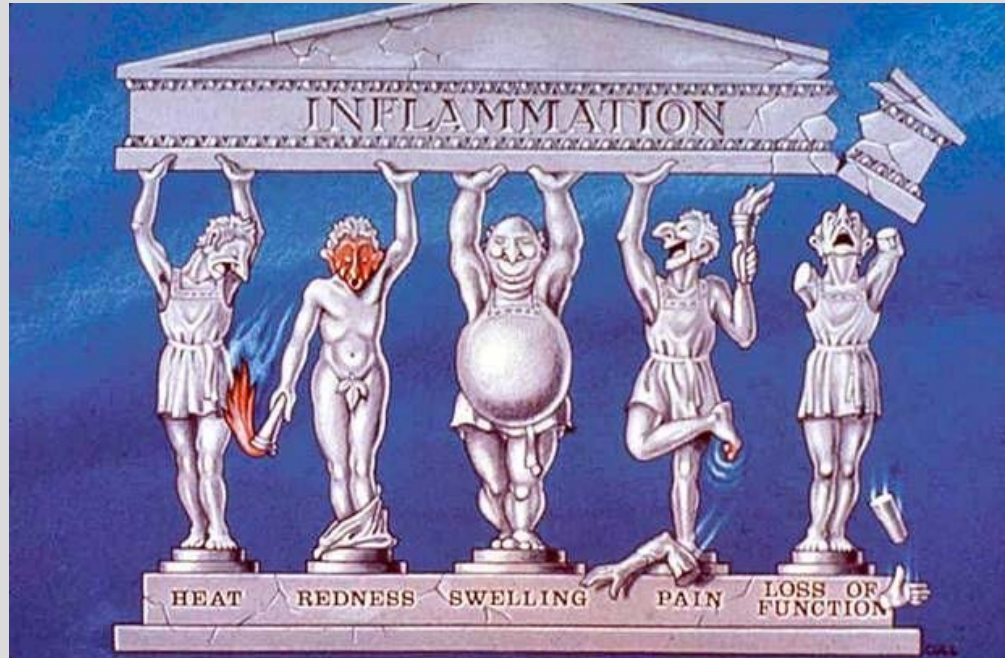
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LOCAL INFLAMMATION  SYSTEMIC FORM


TNF α , IL-1 α/β , IL-6 etc.

IBD

Cancer

Diabetes
II

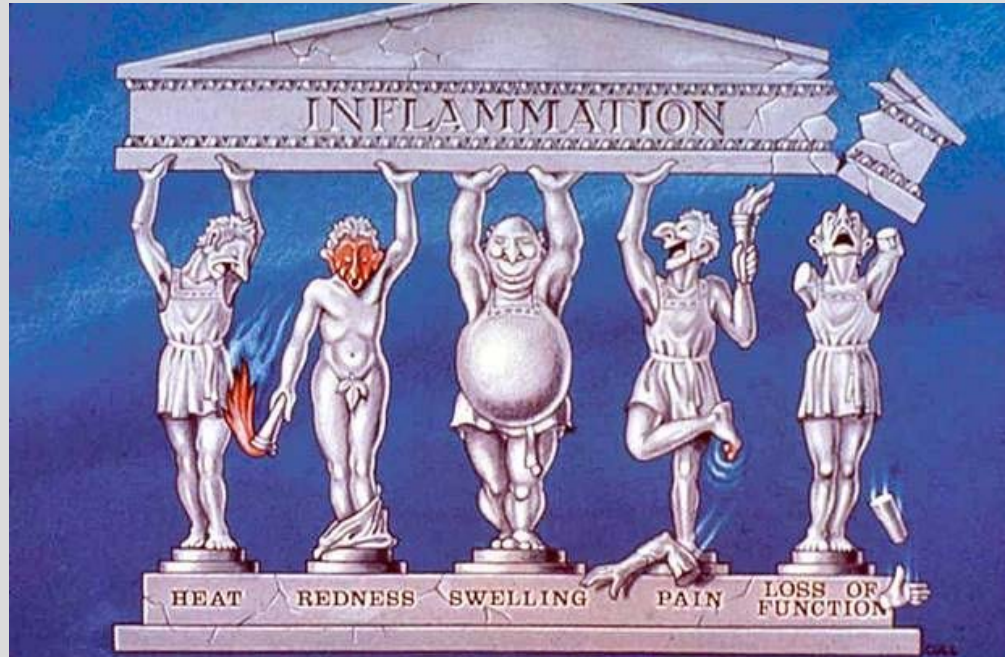
AI

CVD


Arthritis

Alzheimer

Pulmonary
.....



LOCAL INFLAMMATION  SYSTEMIC FORM


TNF α , IL-1 α/β , IL-6 etc.

Treatment of chronic inflammation

- Antibiotics (resistance)
- Corticosteroids (non-specific inhibition of PG)
- Biological drugs
(monoclonal Ab, cytokine-antagonists)
specific effect, high cost, possible adverse effects

Approved drug	Use	2012 Sales (\$ billions)	Originator & major marketers	Patents expire		Biosimilars in development	Major players/partners in biosimilars for regulated markets
				U.S.	EU		
Humira (adalimumab)	Inflammatory diseases	9.3	AbbVie	2016	2018	16	Amgen, BioXpress, Boehringer Ingelheim, Fujifilm Kyowa Kirin Biologics, Pfizer
Remicade (infliximab)	Inflammatory diseases	8.2	Johnson & Johnson, Merck & Co.	2018	2015	9	Amgen, BioXpress, Celltrion/Hospira, Pfizer, Samsung Bioepis
Enbrel (etanercept)	Inflammatory diseases	8.0	Amgen, Pfizer	2019	2015	21	BioExpress, Samsung Bioepis, Sandoz
Rituxan (rituximab)	Cancers, arthritis	7.4	Biogen Idec, Genentech, Roche	2018	2013	34	Amgen/Actavis, Biocad, BioXpress, Boehringer Ingelheim, Pfizer, Sandoz, Stada/Gedeon Richter
Herceptin (trastuzumab)	Cancers	6.5	Genentech, Roche	2019	2015	30	Amgen/Actavis, Biocad, Biocon, BioXpress, Celltrion/Hospira, Pfizer, Stada/Gedeon Richter
Avastin (bevacizumab)	Cancers	6.3	Genentech, Roche	2019	2022	16	Amgen/Actavis, Biocad, BioXpress, Boehringer Ingelheim, Fujifilm Kyowa Kirin Biologics

SOURCES: Company data, Biotechnology Information Institute

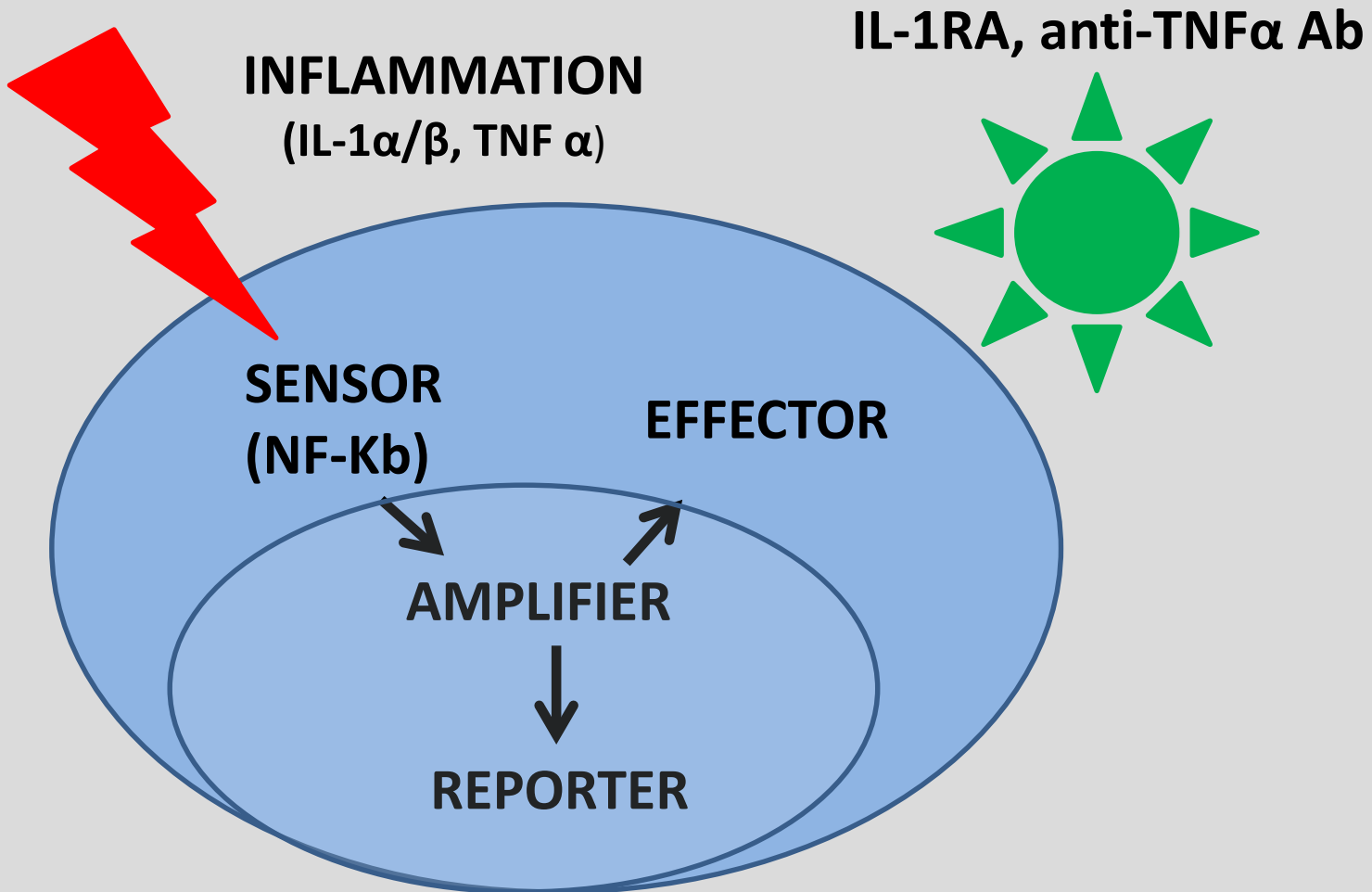
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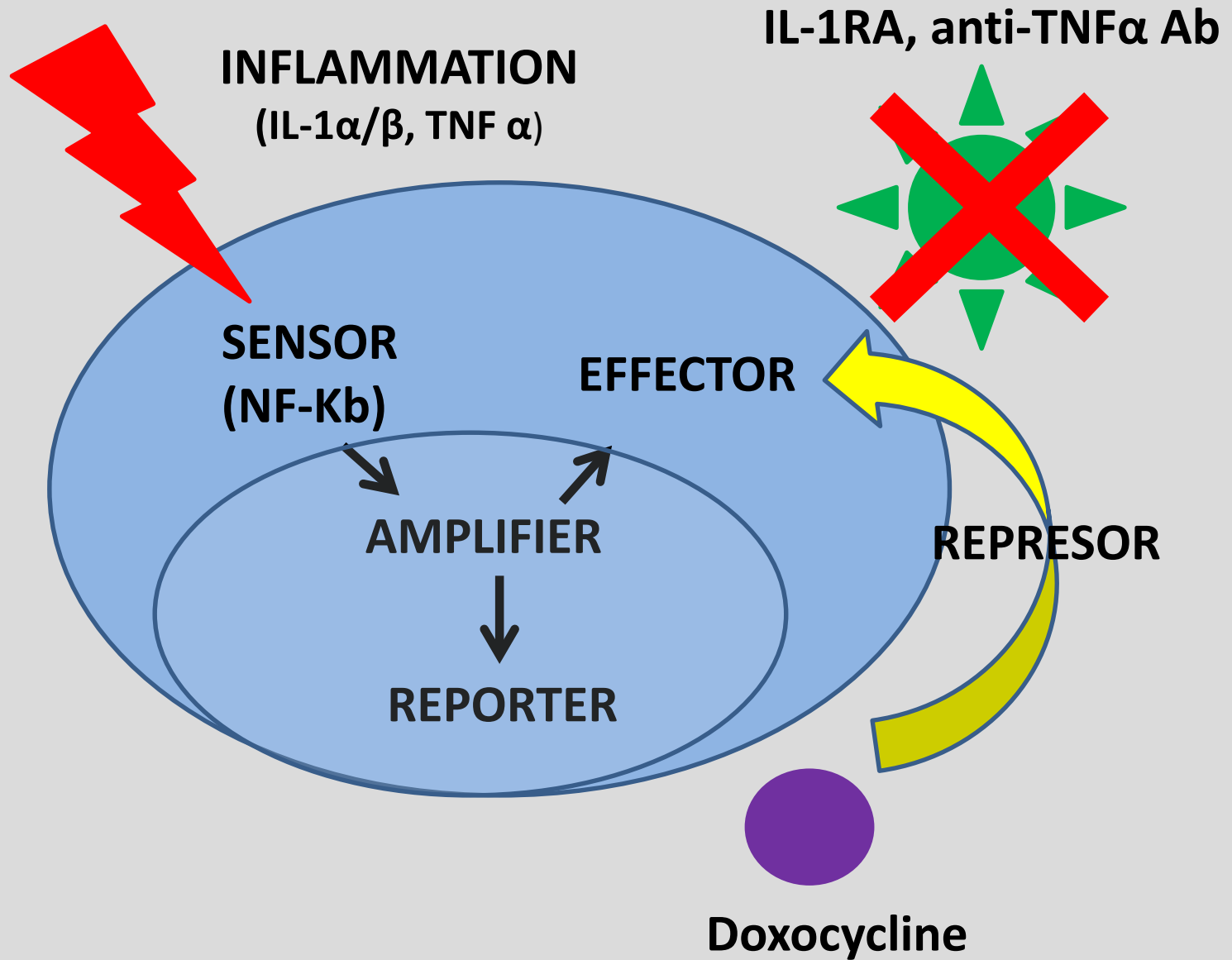
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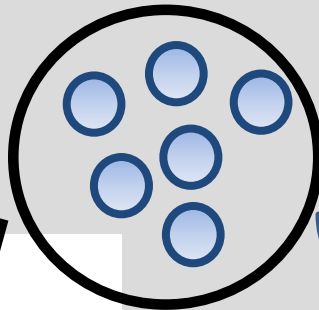
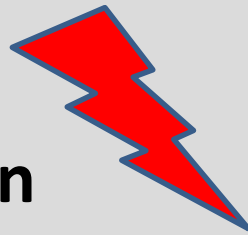
Cell-based therapy

- Development of synthetic biology → design of engineered cells (a prosthetic artificial implant)
- We undertook the challenge to develop a **gene regulated anti-inflammatory device**
 - could act autonomously
 - able to produce a therapeutic proteins





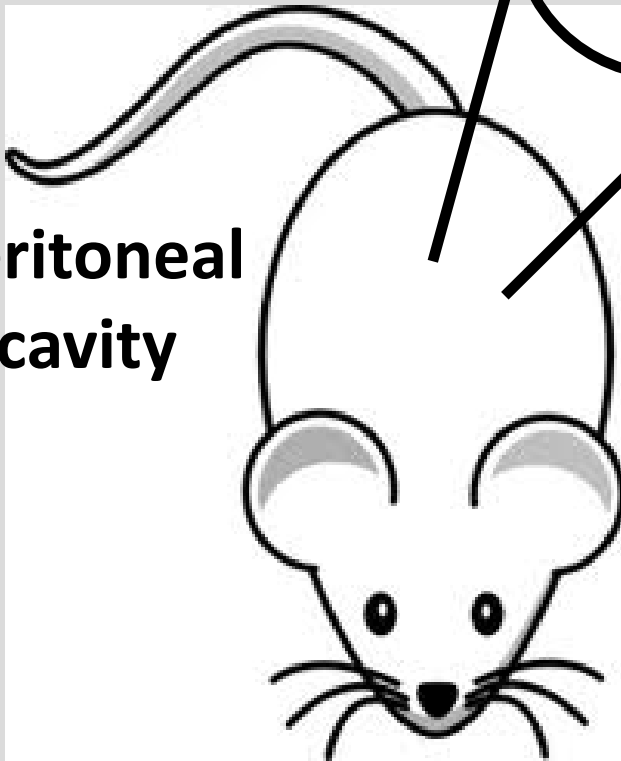
Inflammation

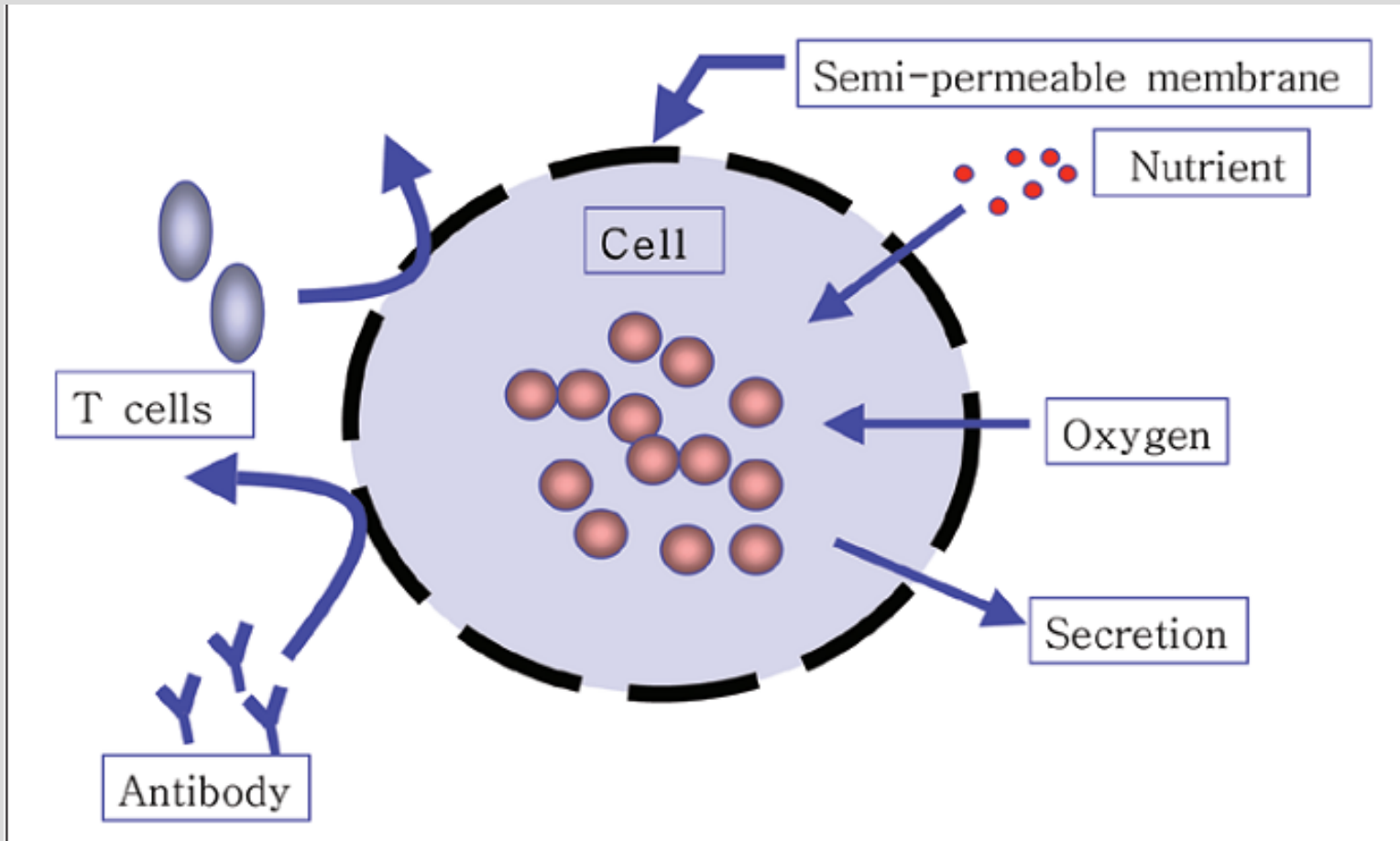


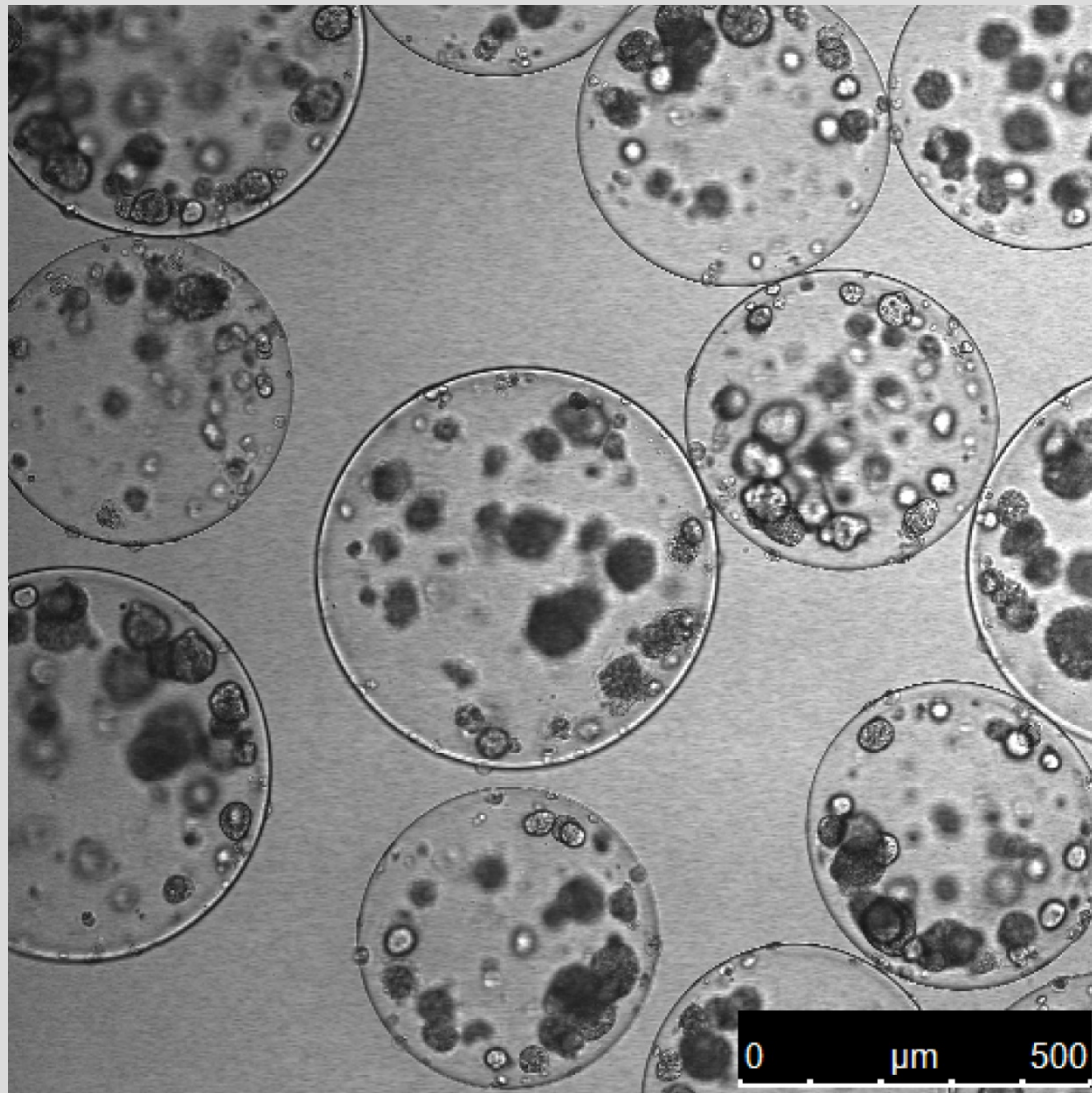
Anti-inflammatory proteins

Encapsulated mammalian cells transfected with anti-inflammatory device

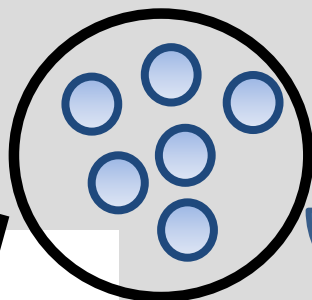
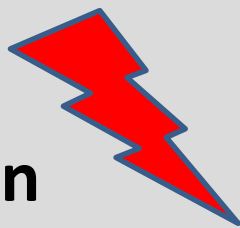
Peritoneal cavity







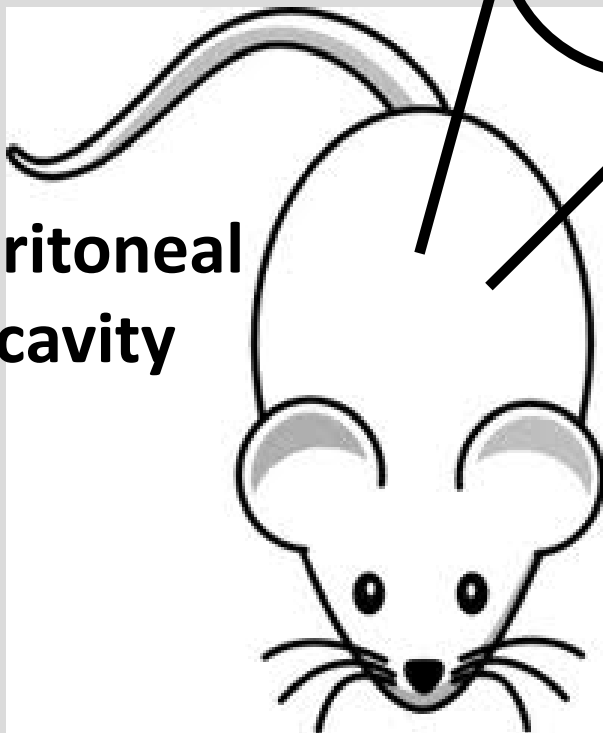
Inflammation



Anti-inflammatory proteins

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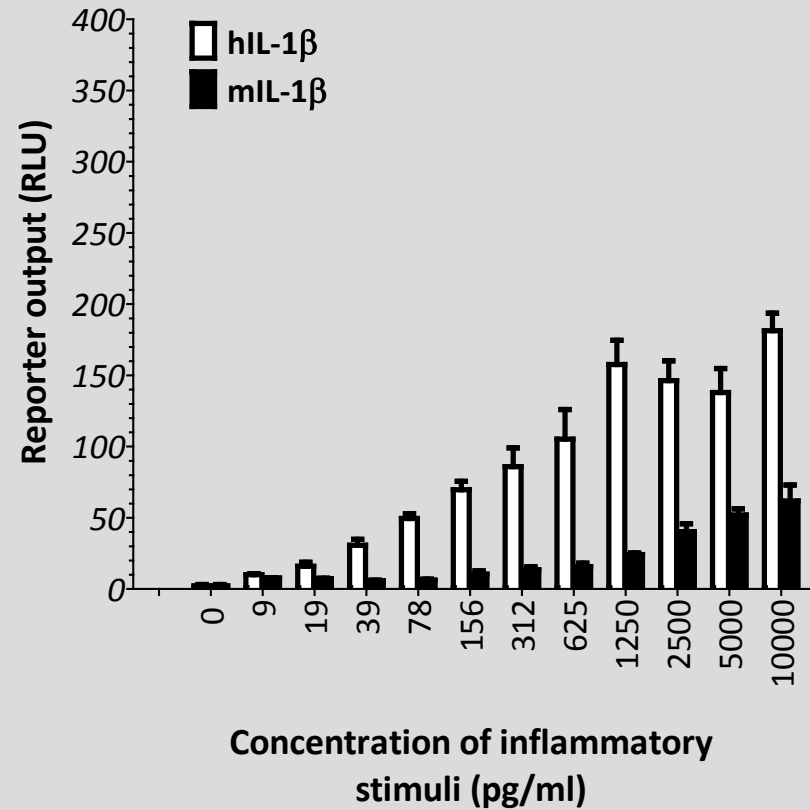
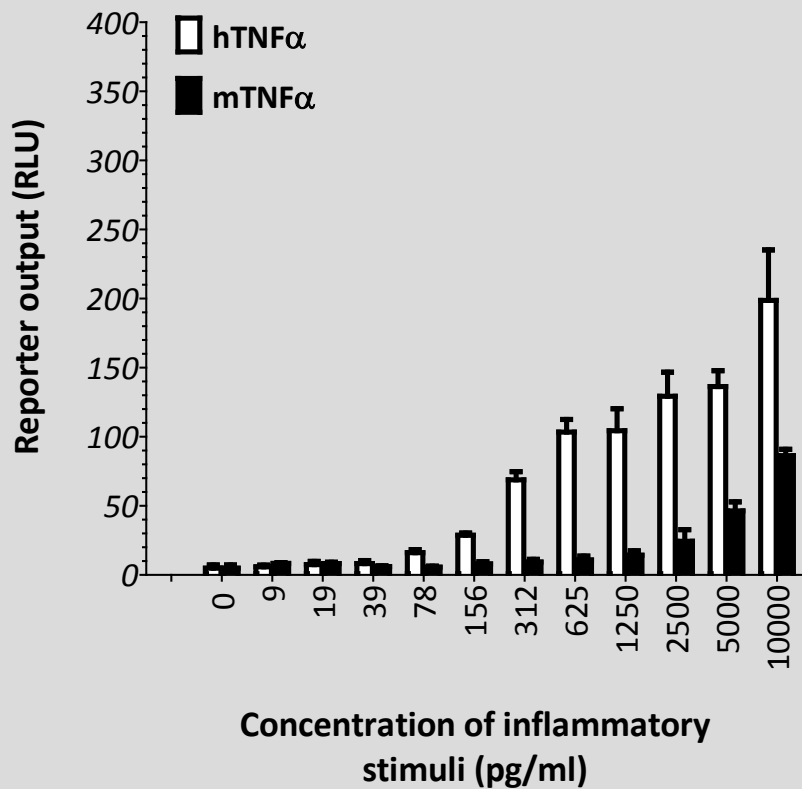
Peritoneal cavity



All animal experiments were approved by The administration of the Republic of Slovenia for food safety, veterinary and plant protection (U34401-55/2013/6).

The responsiveness of the system to an inflammatory signal

HEK293T

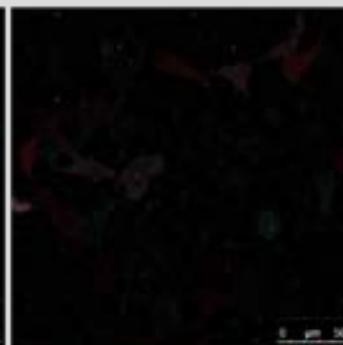
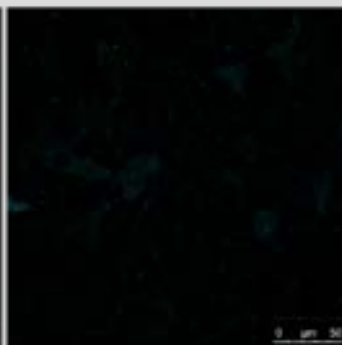
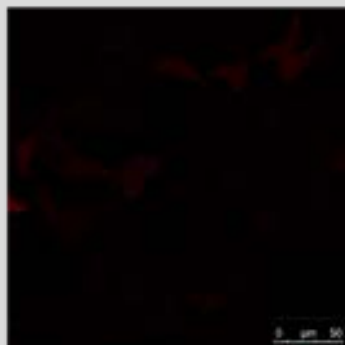


Sensor-mCit

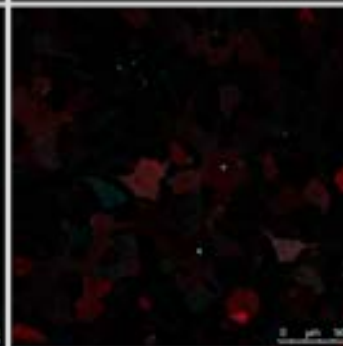
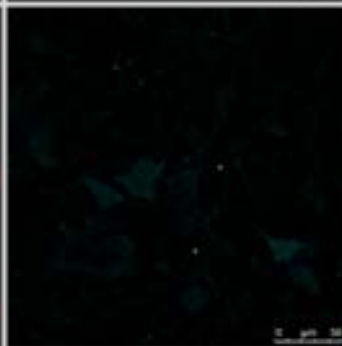
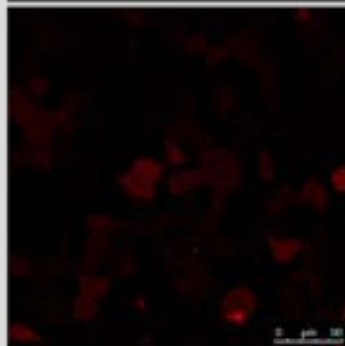
Amplifier-tagBFP

Overlay

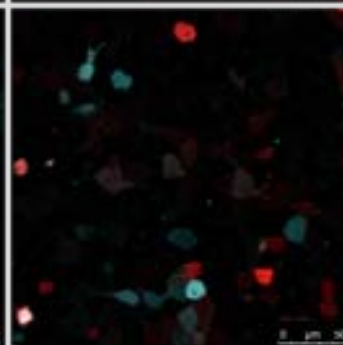
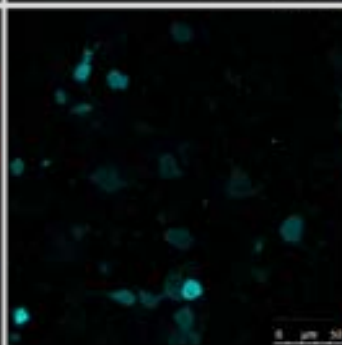
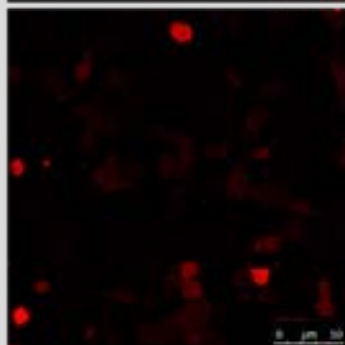
S(0 ng)+A(0 ng)

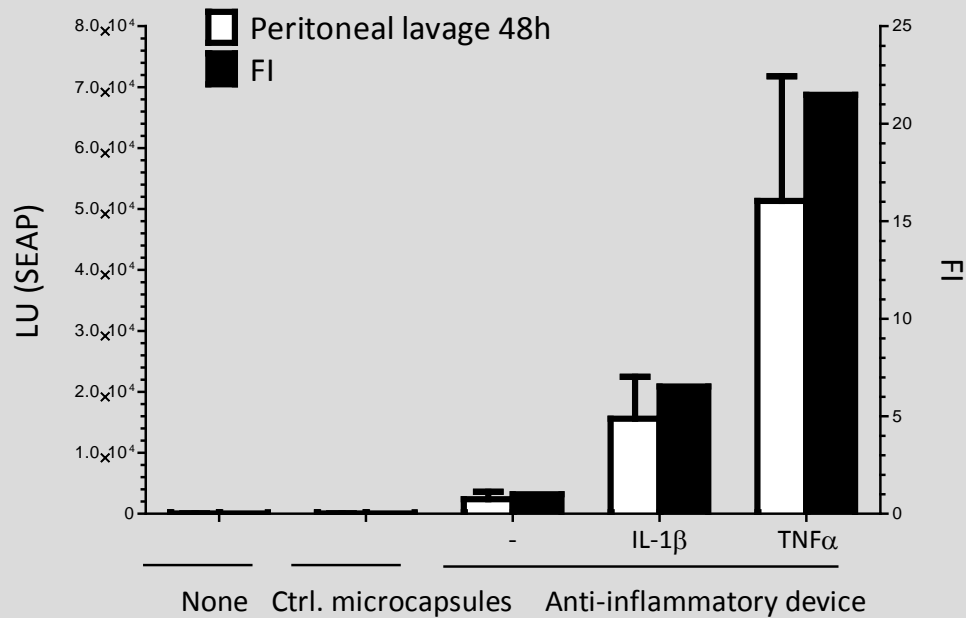
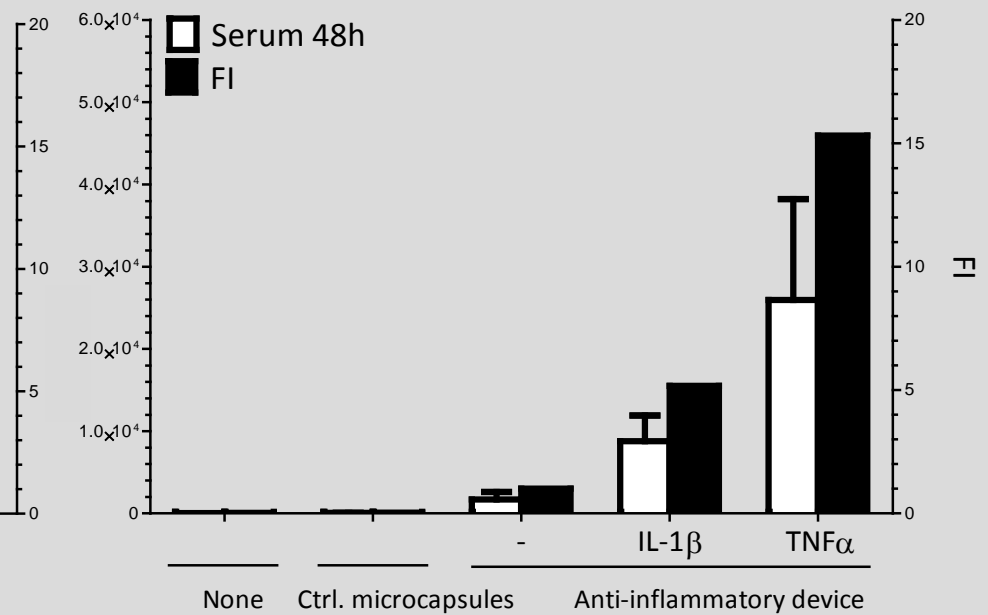
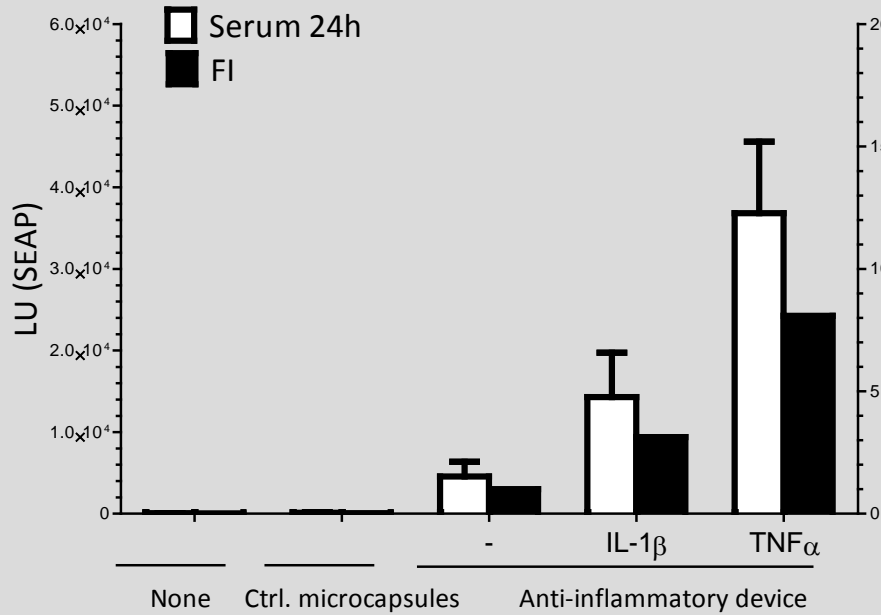


S(1 ng)+A(0 ng)



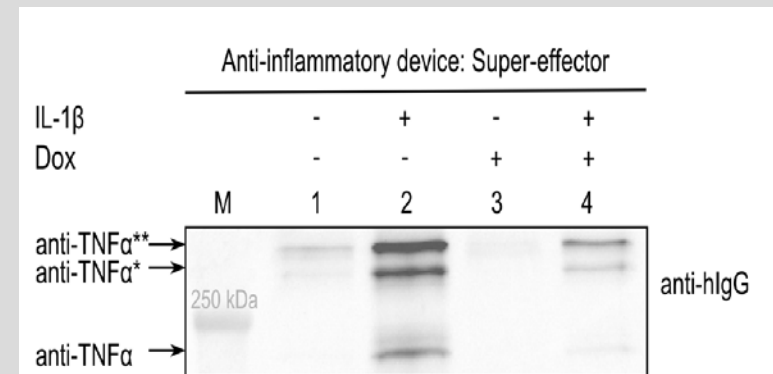
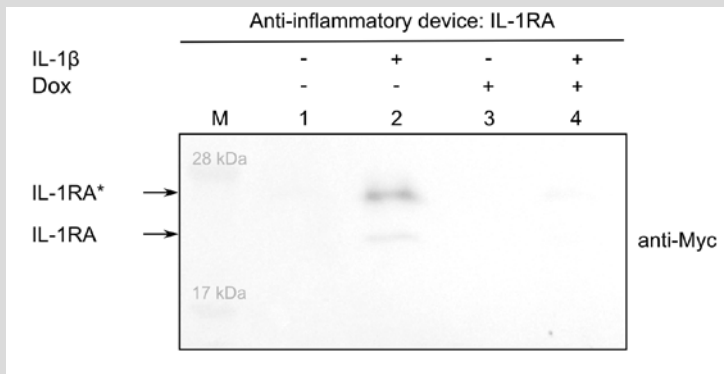
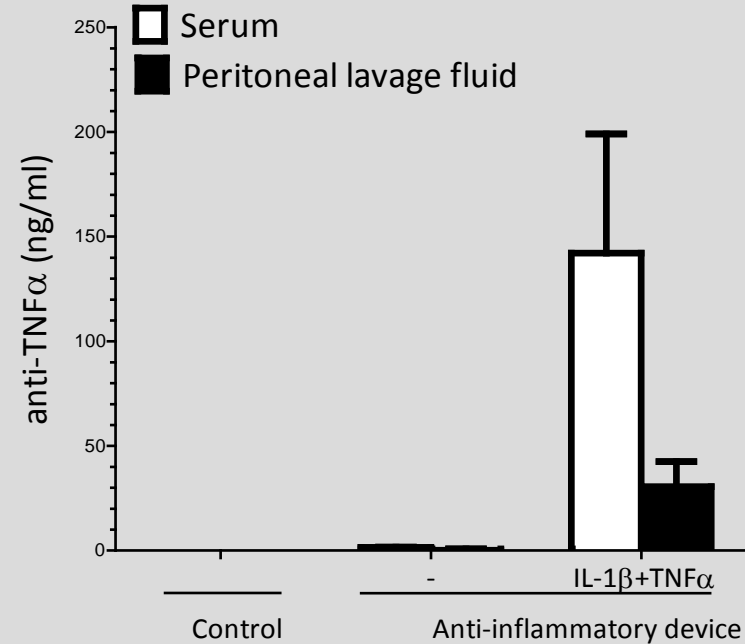
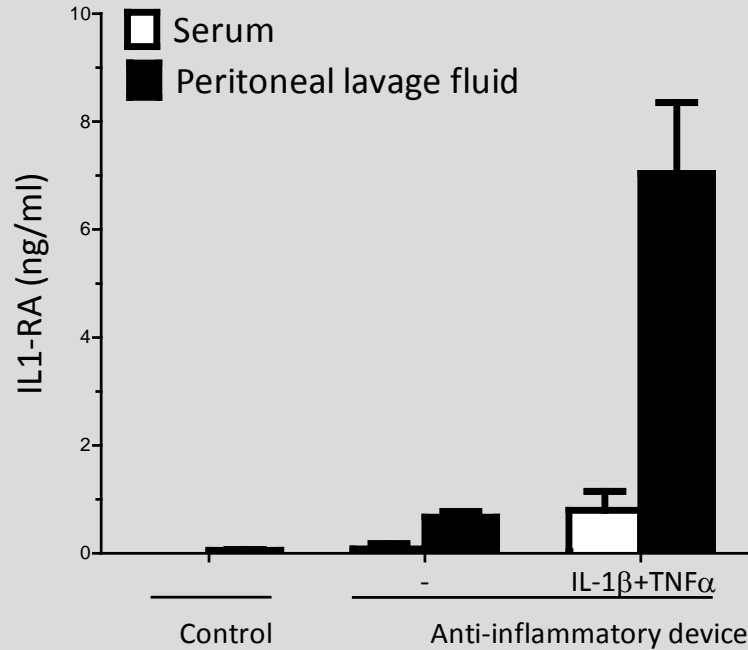
S(1 ng)+A(1 ng)





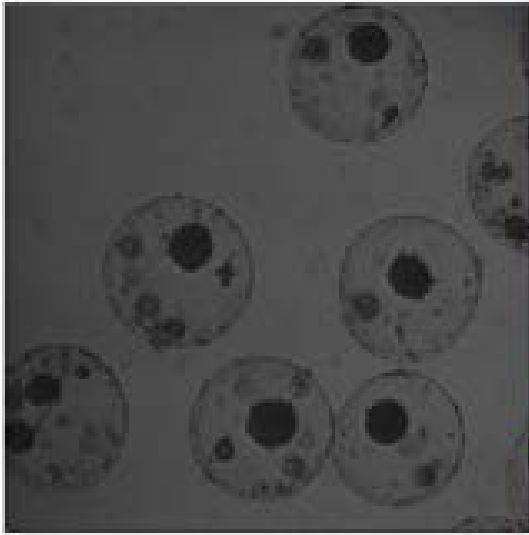
C57Bl6/OlaHsd

Production of the anti-inflammatory proteins

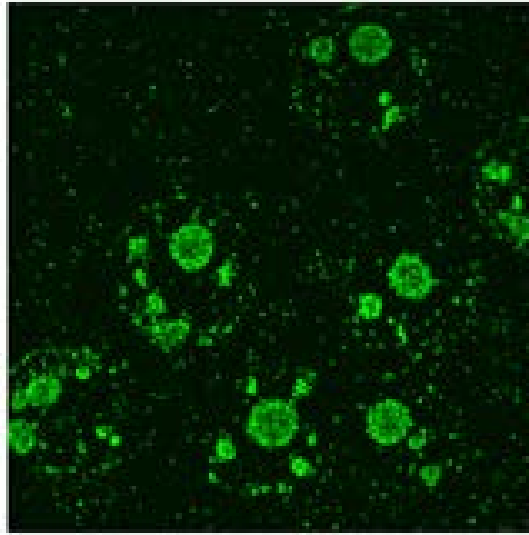


Cells after 3 days in the animals

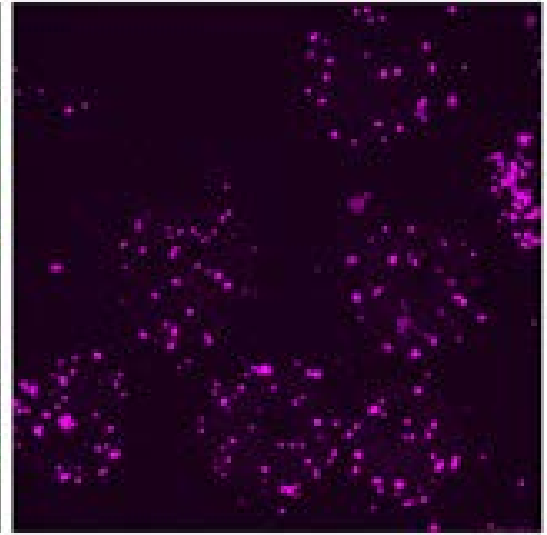
Bright field



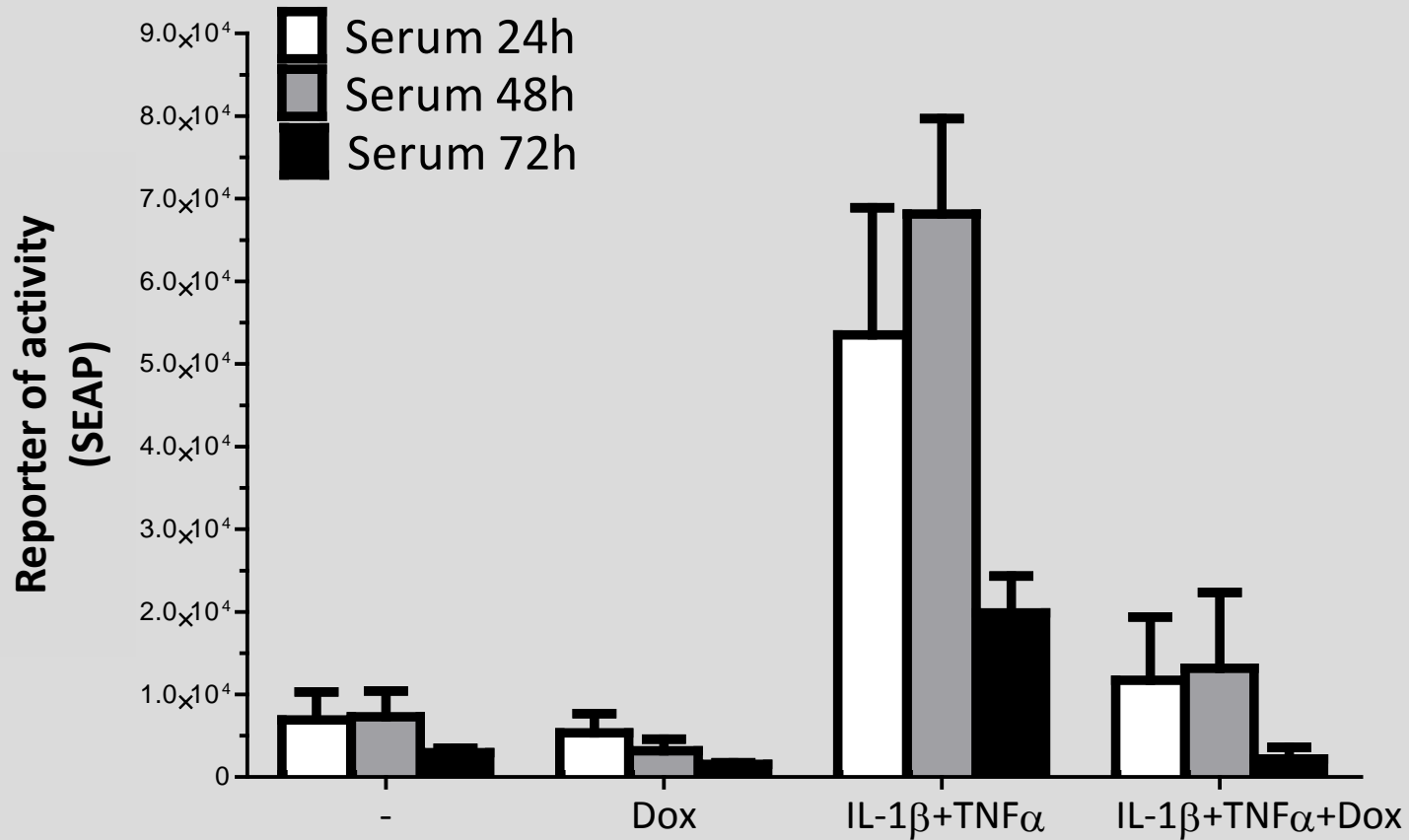
**Live cells
(Hoechst)**



**Dead cells
(PI)**



The reversibility of the system („off-state“)



Conclusions

- We developed a synthetic regulatory anti-inflammatory device in mammalian cells.
- We showed that the device is silent in the absence of inflammation.
- The device can be reset to off state via doxycycline.

Acknowledgments



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- prof. dr. Horvat
- Anže
- Urban

- doc.dr. Tatjana Pirman

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Vesna Mrak for the mouse help



Thank you!!!