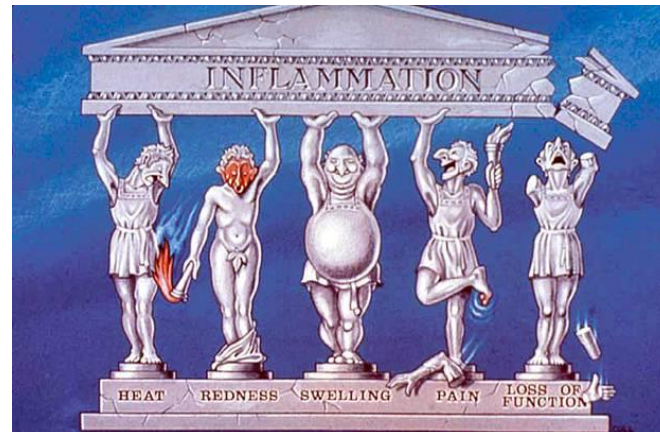


The potential of non-invasive in vivo imaging of inflammatory processes

Manfred Kneilling

Redness (Rubor)
Heat (Calor)
Swelling (Tumor)
Pain (Dolor)
Loss of Function



- ✓ Immune Reactions (hypersensitivity reactions, autoimmune diseases)
- ✓ Infections (pathogens: bacteria, viral, fungal, parasitic)
- ✓ Toxins, Chemical irritants, Ionizing radiation (CT!), Foreign bodies, Burns, Frostbite, Trauma.....

A protective response to:

- ✓ eliminate the initial cause of cell injury
- ✓ remove necrotic cells and tissues
- ✓ initiate the process of repair

Normally self limited but can also be harmful



- Vasodilation: increasing the oxygen supply
- Accumulation of leukocytes (rolling, adhesion, transmigration)
- Bradykinin, Histamine and Leukotrienes cause endothelial cell contraction
- Increases vascular permeability (!!!)
- Increase of the lymphatic flow



Involved cells:

- Granulocytes (Neutrophil, Eosinophil, Basophil)
- Monocytes/Macrophages
- Lymphocytes (T and B)
- Platelets
- Mast cells
- Dendritic Cells
- Fibroblasts

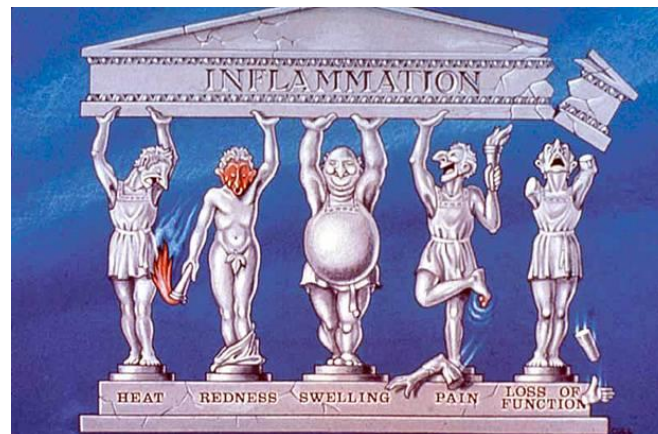
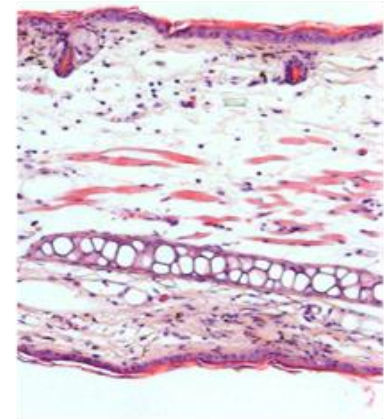
Involved proteins:

- Complement
- Proteoglycans
- Kinines/Cytokines/Chemokines....



Classical signs of acute inflammation:

- Dolor (pain),
- Calor (heat),
- Rubor (redness),
- Tumor (swelling),
- Functio laesa (loss of function)



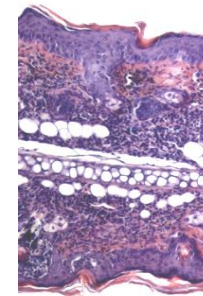
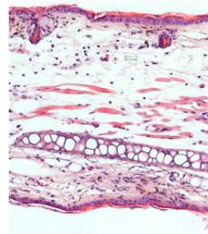


Vasodilatation and causes an increased blood flow resulting in **redness** (*rubor*) and increased heat (*calor*). The increased permeability of the blood vessels causes **exudation** (leakage) of plasma proteins and fluid into the tissue (**edema**), which manifests itself as **swelling** (*tumor*). Some of the released mediators such as bradykinin increase the sensitivity to **pain** (hyperalgesia, *dolor*).



Inflammation can be classified as either Acute or Chronic:

	Acute	Chronic
Onset:	fast (minutes - hours)	slow (days)
Cellular infiltrate:	predominantly neutrophils	monocytes/macrophages and lymphocytes
Tissue injury/damage:	normally mild and self-limited	often severe, progressive and irreversible destruction

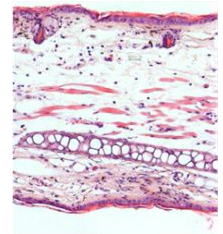


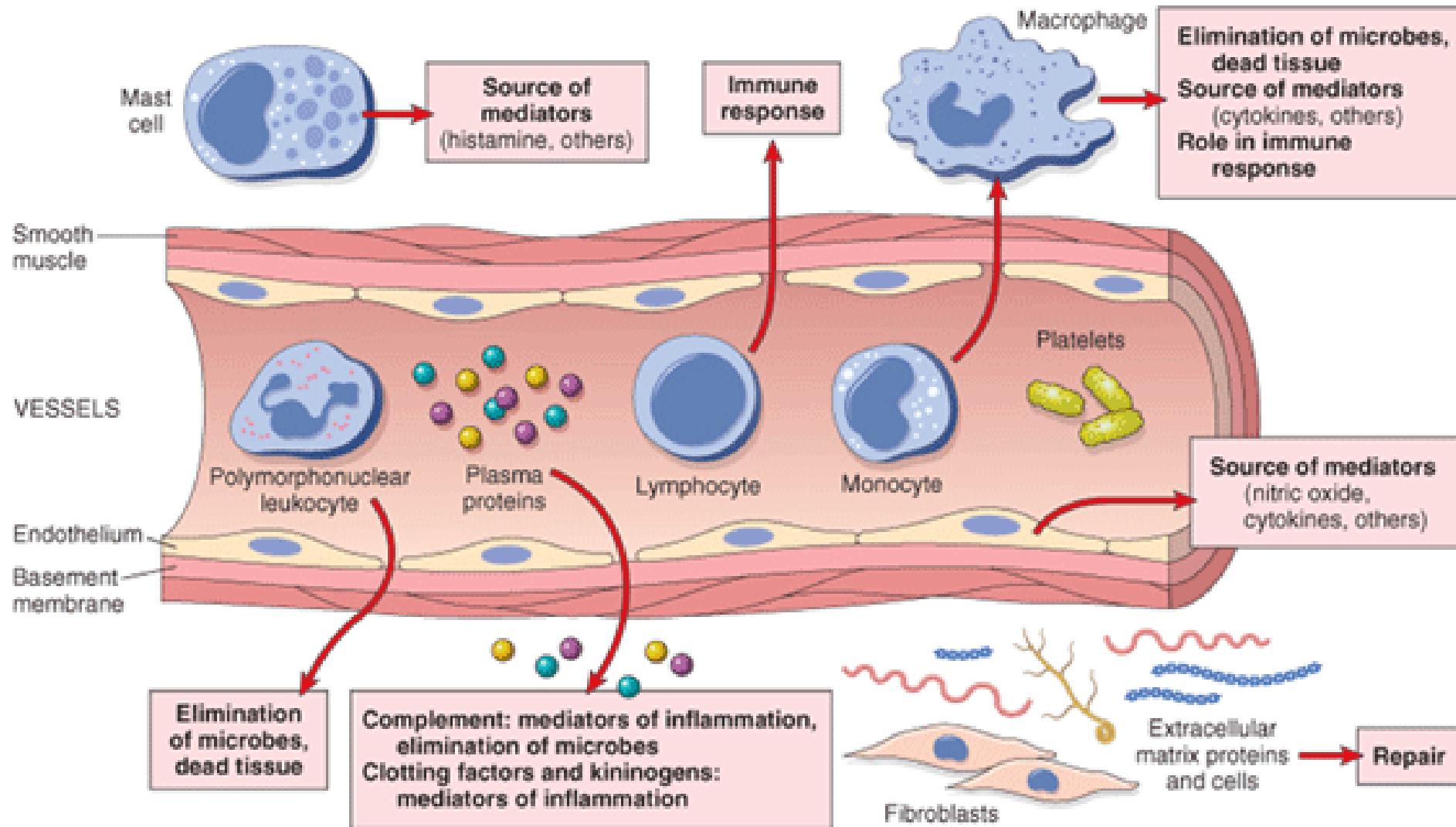


Vascular:

vasodilatation, vascular permeability (accumulation of fluid/plasma in the extra-vascular tissues;
early transudate (low protein content;
exudates (high protein content)),

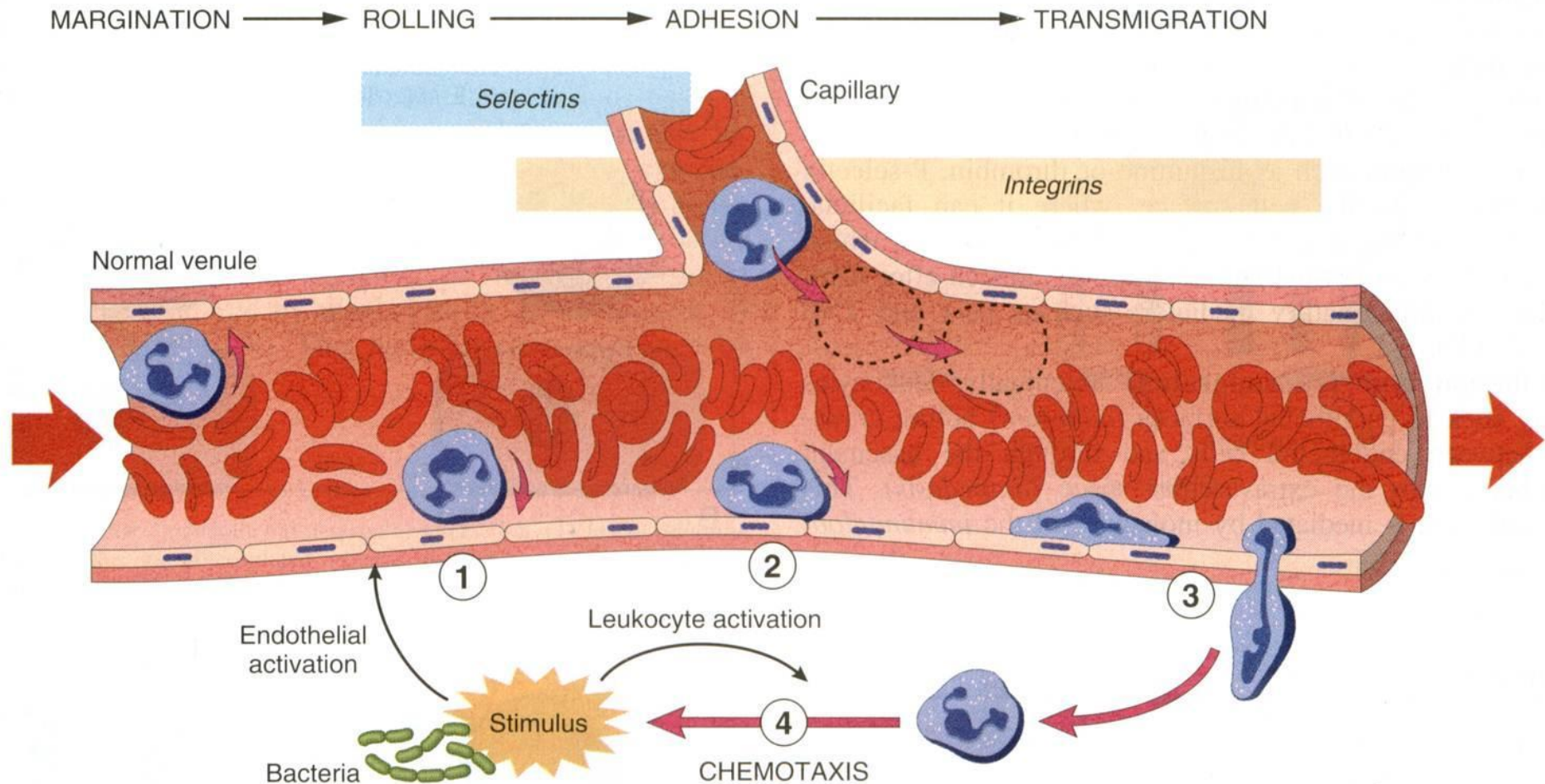
endothelial activation, increased expression of adhesion molecules, chemotaxis, increased rolling/adhesion/transmigration of activated leukocytes emigration to the extravascular tissues





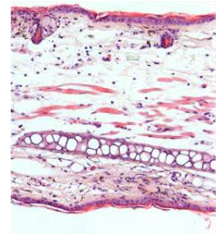
C. Wall et al. Immunology 2012

Rolling – Adhesion - Transmigration





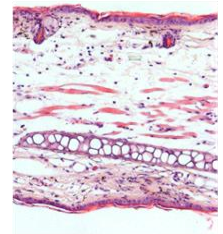
intra-vital fluorescence microscopy



Kneilling et al. Blood 2009

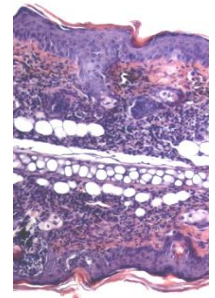


- Cellular: recruitment and activation of neutrophils and leukocytes
- Tissue: edema; normally no severe tissue injury and scar formation
- Example: acute contact hypersensitivity reaction of the skin
toxic shock syndrome,
acute appendicitis;
bacterial broncho pneumonia





- Vascular:** endothelial activation, angiogenesis,
expression of adhesion molecules, chemotaxis;
increased rolling/adhesion/transmigration of
activated leukocytes;
leukocyte emigration
- Tissue:** persistent tissue injury, tissue
destruction by inflammatory cells,
repair with fibrosis/scar formation
cancer induction possible



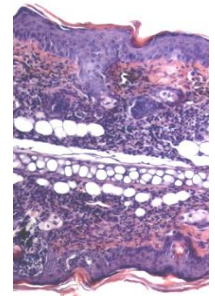


Cellular: mononuclear cell infiltrate (monocytes/macrophages, T- and B- lymphocytes, plasma cells). Thus, the prolonged inflammation, leads to a shift in the type of cells present at the site of inflammation

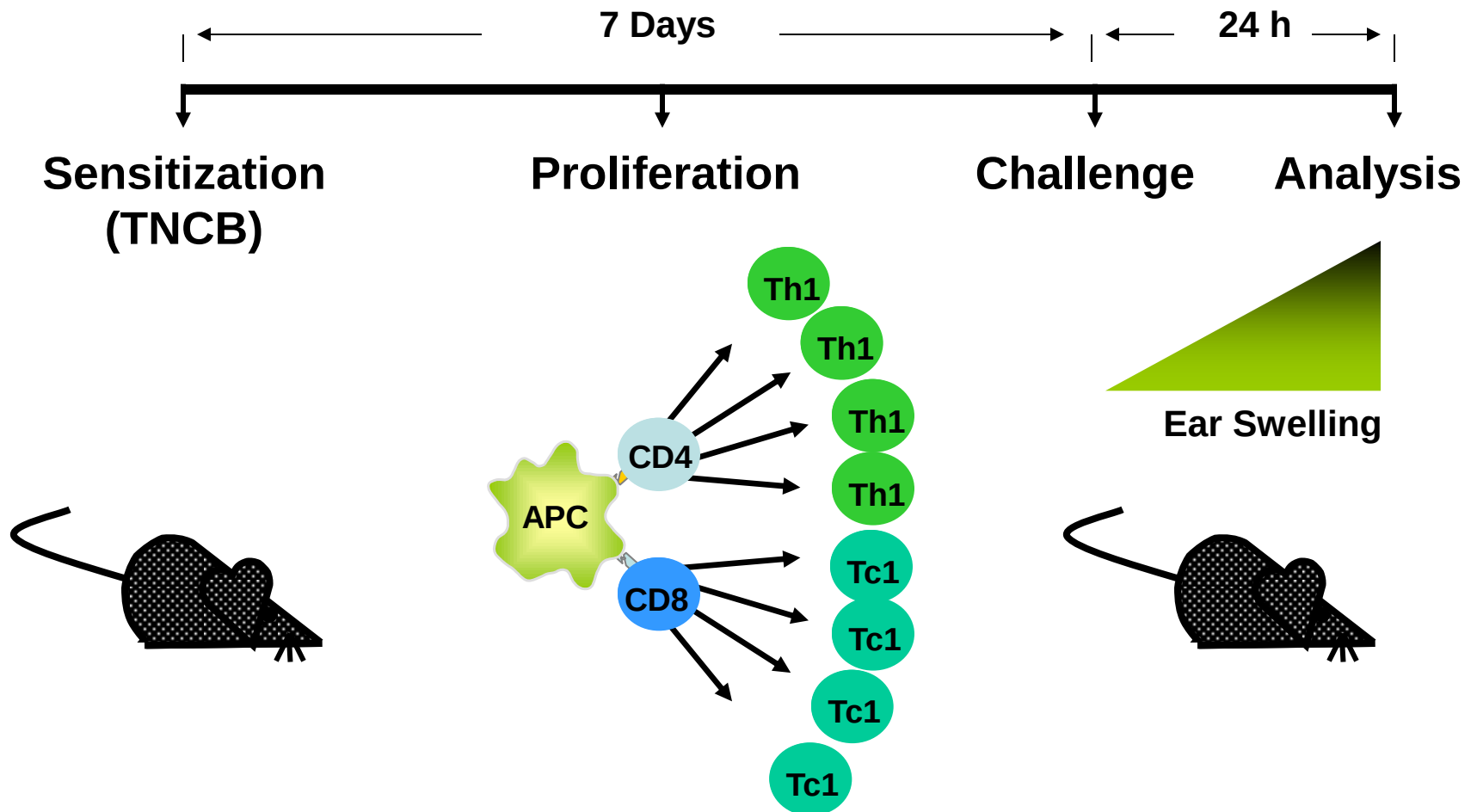
Example: chronic contact hypersensitivity reaction of the skin (Nickel)

Autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis)

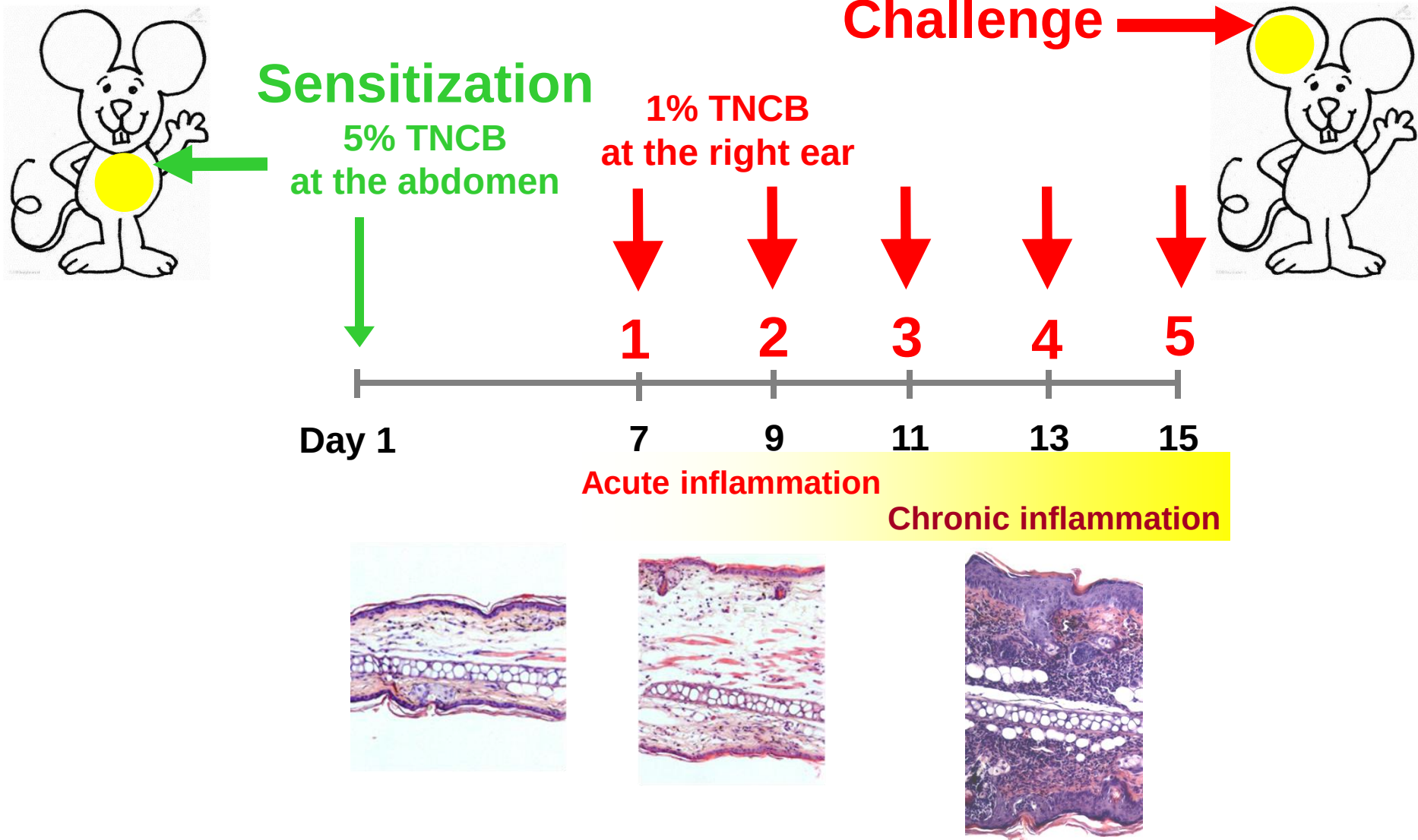
pulmonary silicosis
skin ulcers



Contact Hypersensitivity Reaction

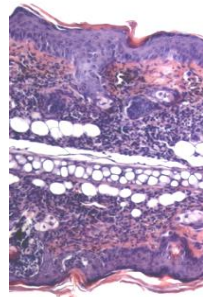
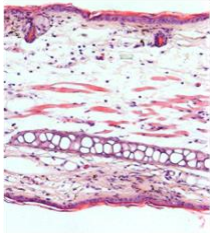
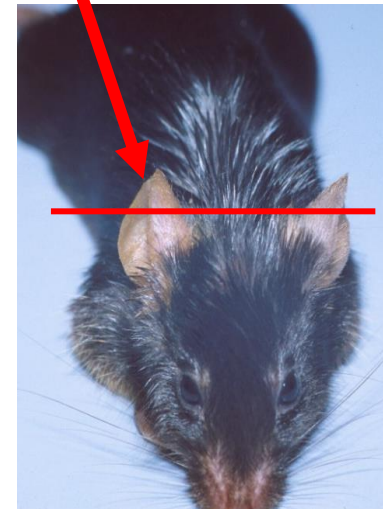
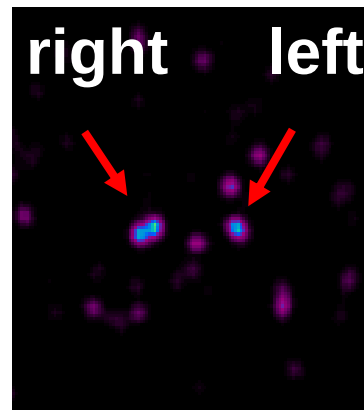
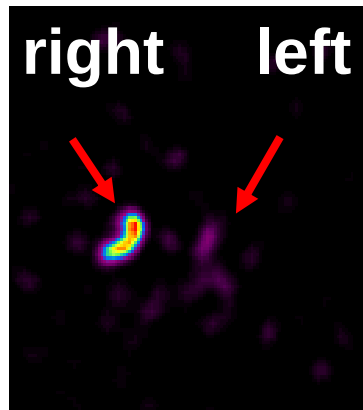
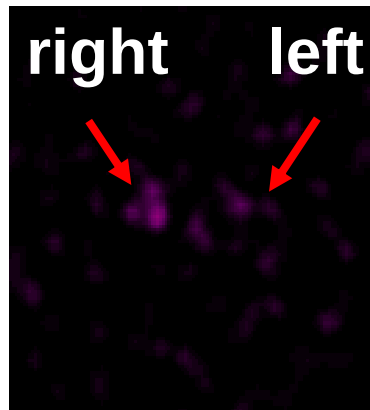


Contact Hypersensitivity Reaction



Schwenck et al. Molecular Imaging 2014

Angiogenesis: [^{18}F]-Galacto-RGD-PET



C-CHSR
blocked with
0.4 mg unlabeled
c(RGDfV)



Imaging of Delayed-Type Hypersensitivity Reaction by PET and ^{18}F -Galacto-RGD

Bernd J. Pichler, PhD¹; Manfred Kneilling, MD²; Roland Haubner, PhD¹; Heidi Braumüller, PhD²; Markus Schwaiger, MD¹; Martin Röcken, MD²; and Wolfgang A. Weber, MD¹

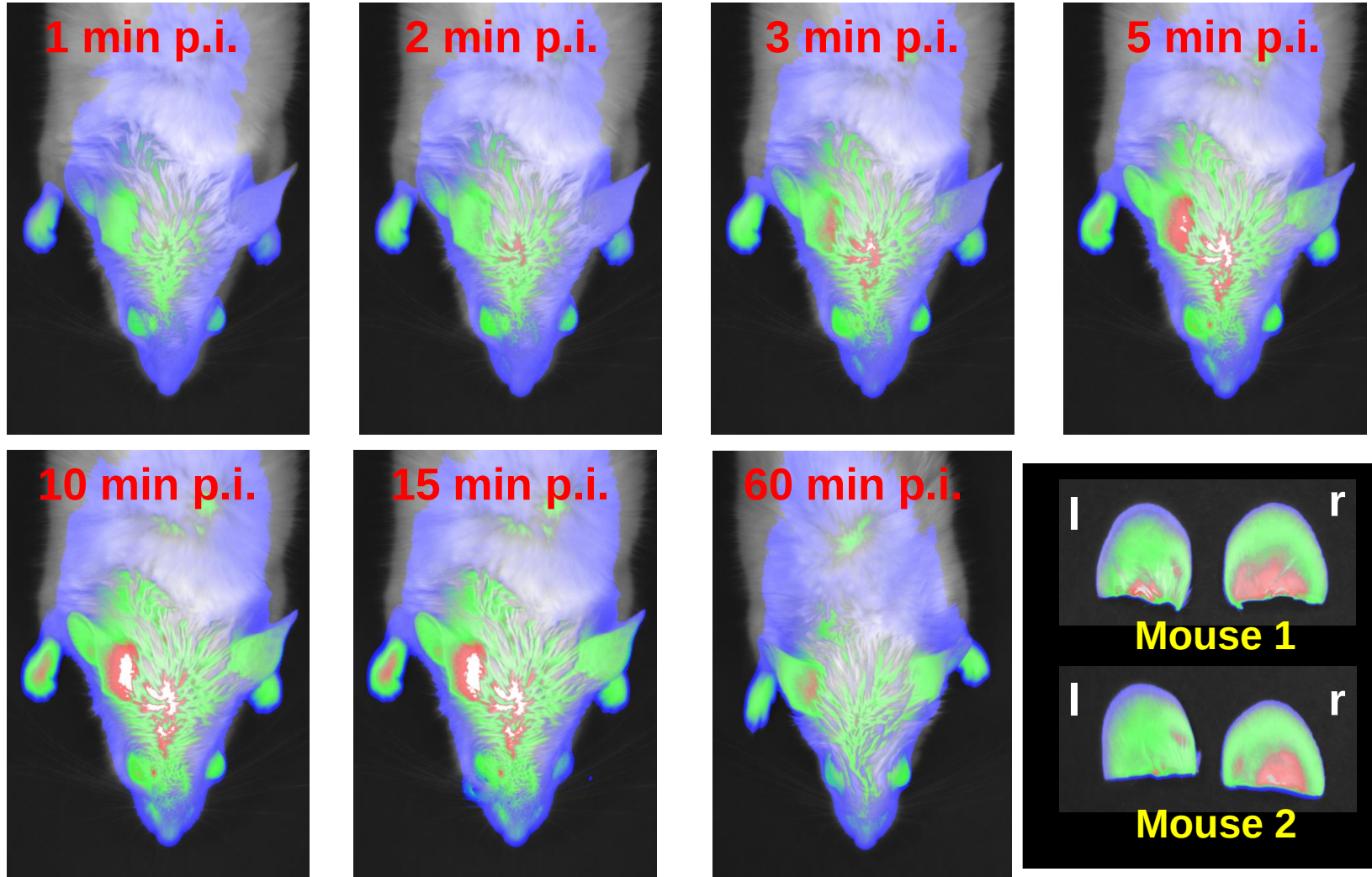
¹Nuklearmedizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, München, Germany; and ²Department of Dermatology, University Tübingen, Tübingen, Germany

Radiolabeled cyclic peptides containing the amino acid sequence arginine-glycine-aspartate (RGD peptides) have successfully been used to image the expression of the $\alpha_v\beta_3$ integrin in malignant tumors. However, the $\alpha_v\beta_3$ integrin also plays an

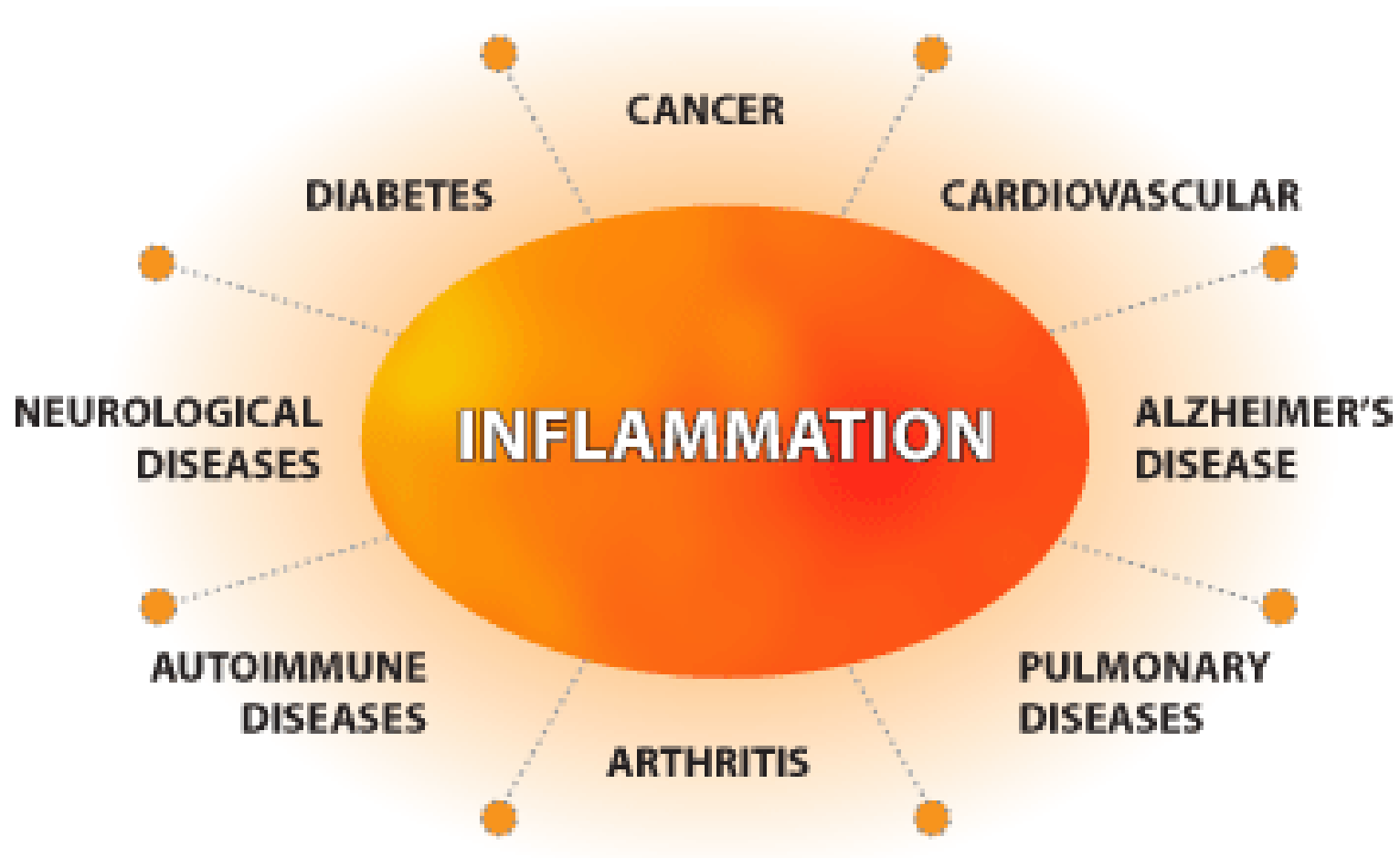
Key Words: autoradiography; molecular imaging; PET; radio-pharmaceuticals; animal imaging; inflammation

J Nucl Med 2005; 46:184–189

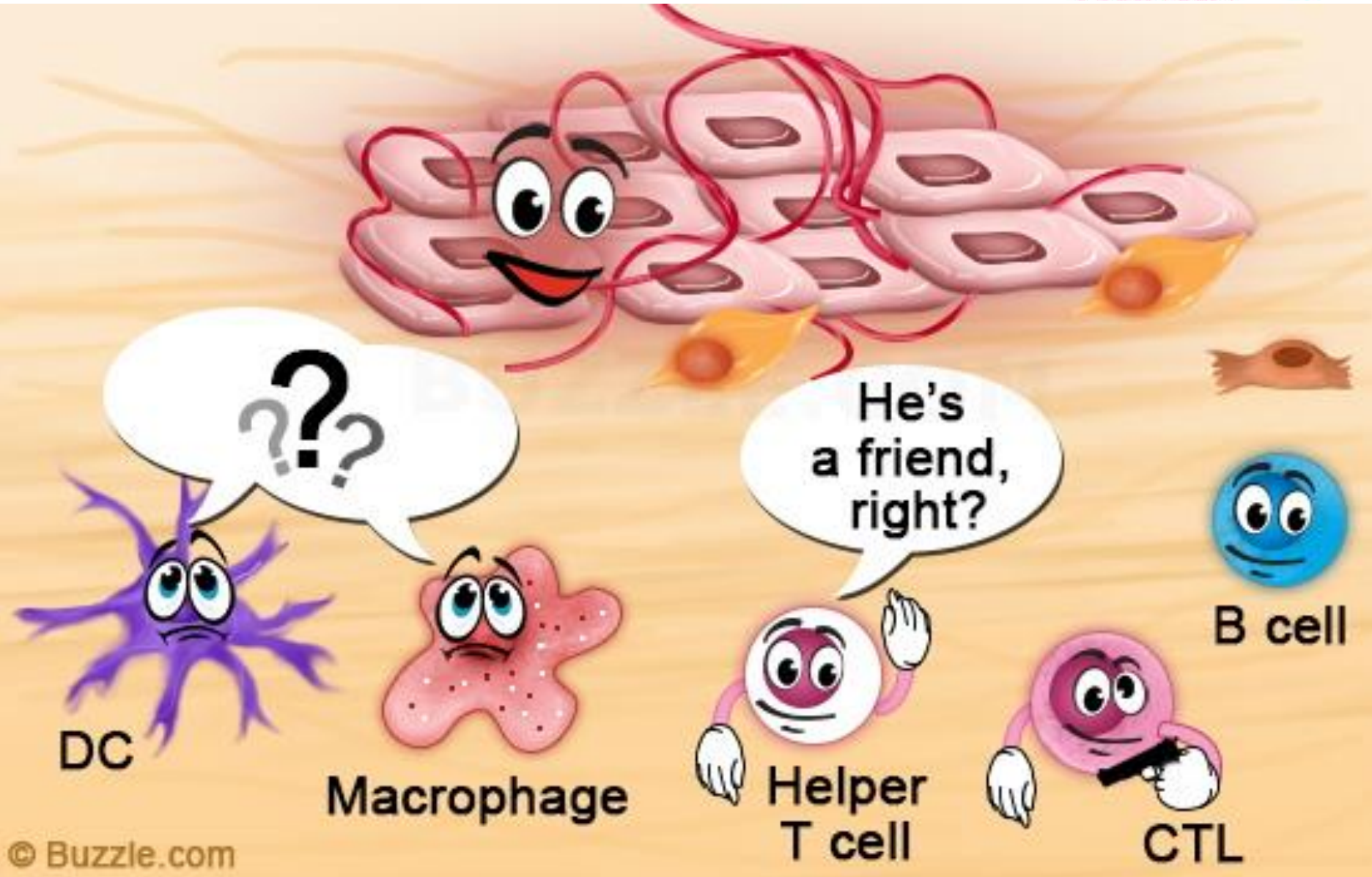
Angiogenesis: Cy5-Galacto-RGD Optical Imaging



The golden standard in nuclear medicine imaging of inflammation is the use of autologous radiolabeled leukocytes ($[^{111}\text{In}]$ -oxine-SPECT)

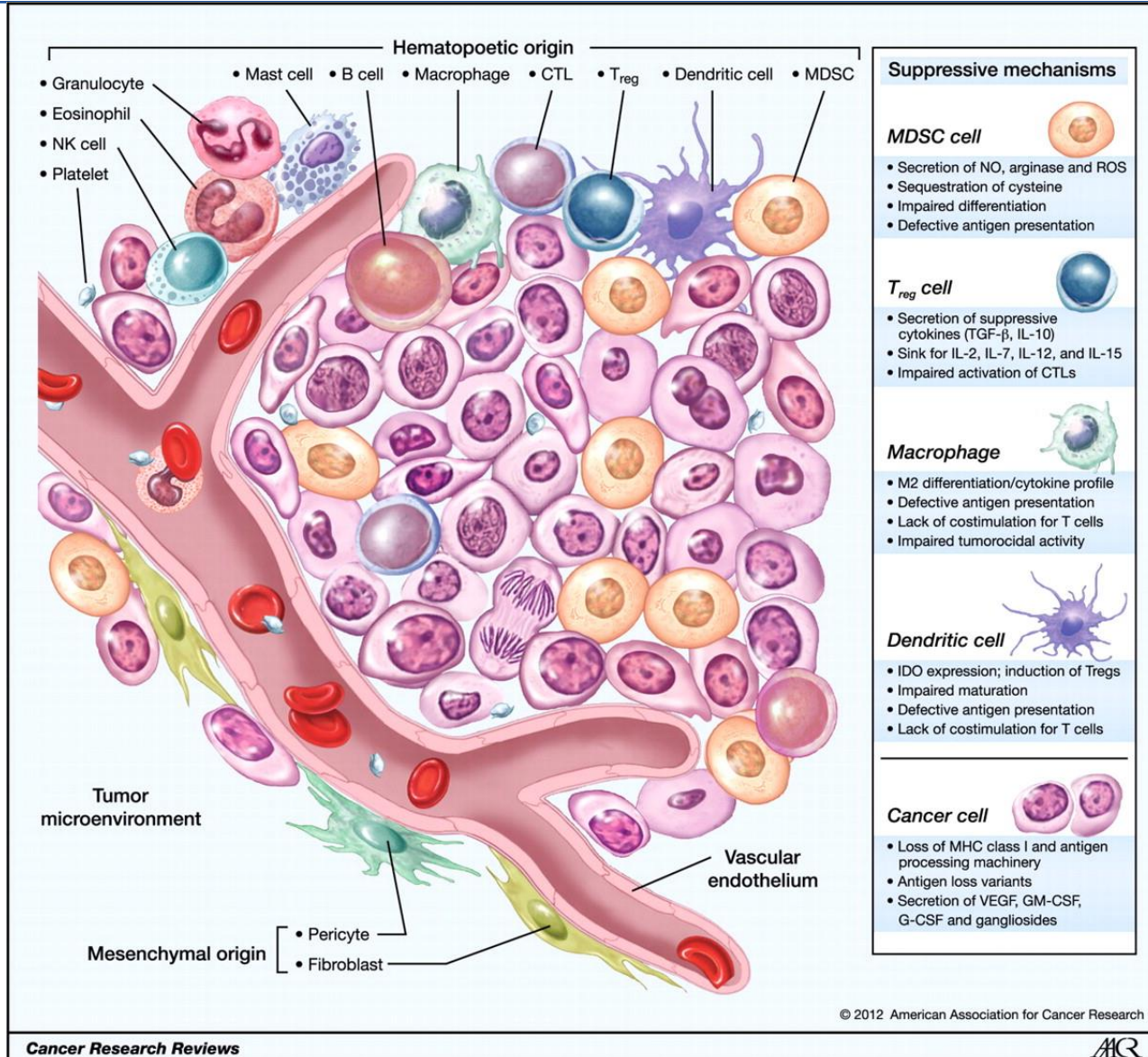


Tumor Microenvironment

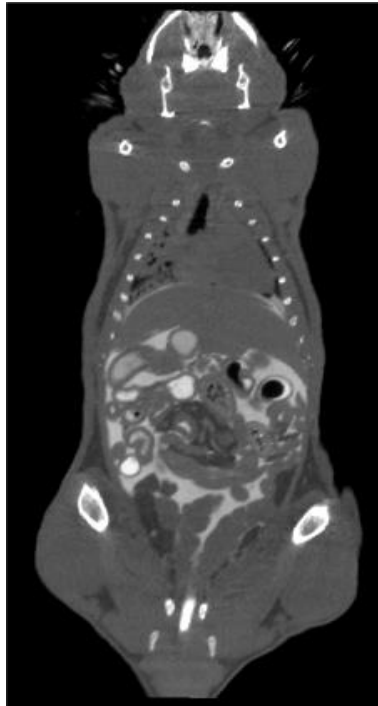


© Buzzle.com



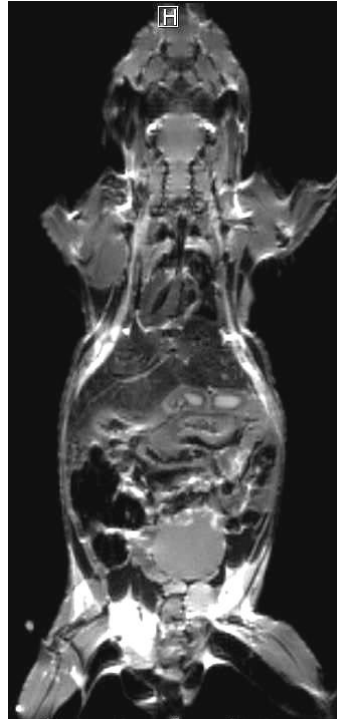


CT



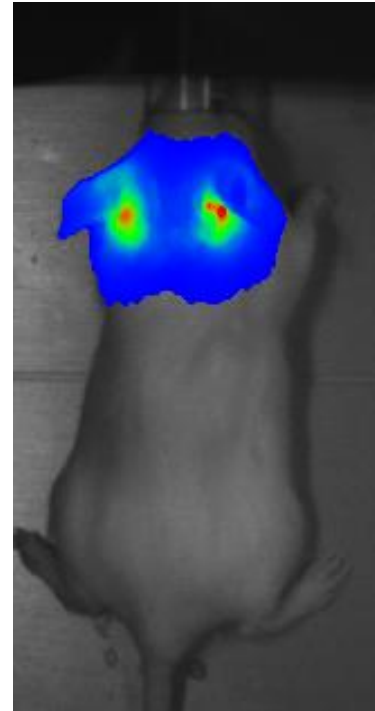
Morphology
(Radiation!)

MRI



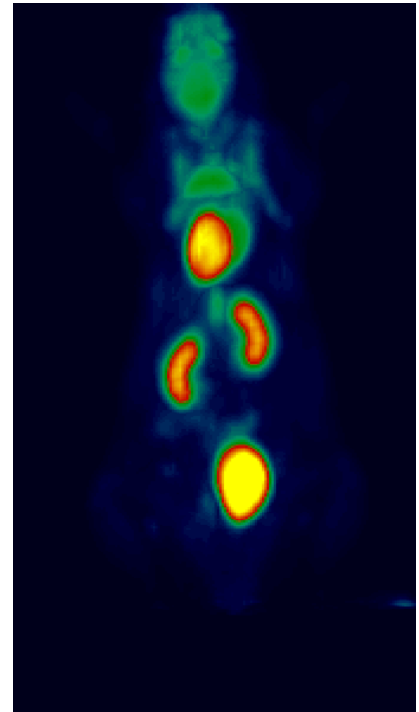
Morphology
(Function)

**Optical
Imaging**



Function

PET



Function

Imaging modalities for non invasive in vivo imaging of inflammation:

Positron emission tomography (PET)

- function, metabolism

Single-Photon Emission Computed Tomography (SPECT)

- function, metabolism

Optical Imaging (OI)

- function, metabolism

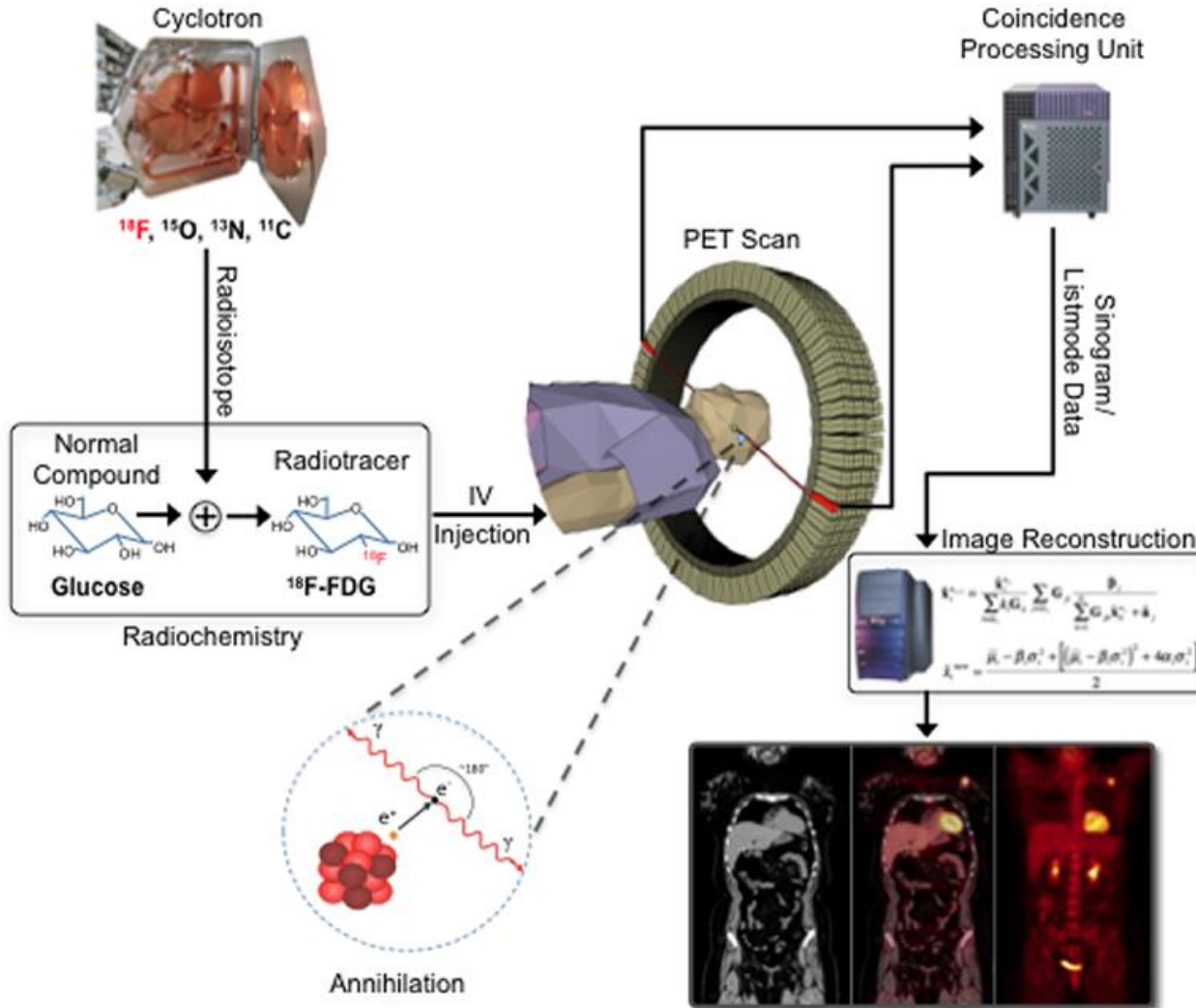
Magnetic Resonance Imaging (MRI)

- anatomy, (function, perfusion)

Computed Tomography (CT)

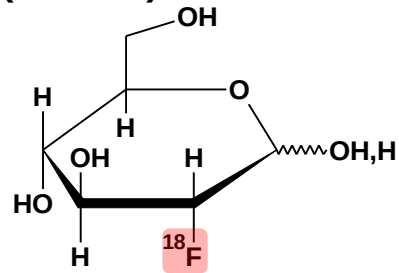
- anatomy, (perfusion)

Positron Emission Tomography

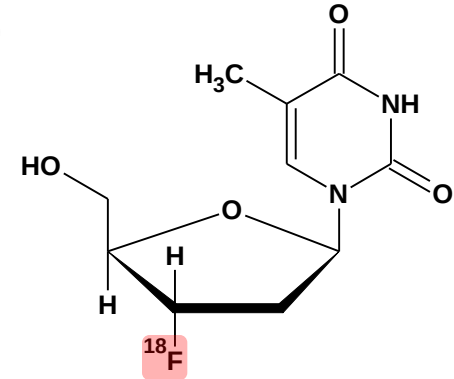




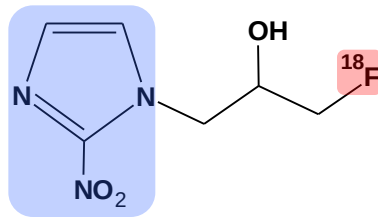
[¹⁸F]FDG: [¹⁸F]Fluorodeoxyglucose:
Glucose metabolism (GLUT-1)



[¹⁸F]FLT: 3'-Deoxy-3'-[¹⁸F]fluorothymidine:
Proliferation (TK-1)

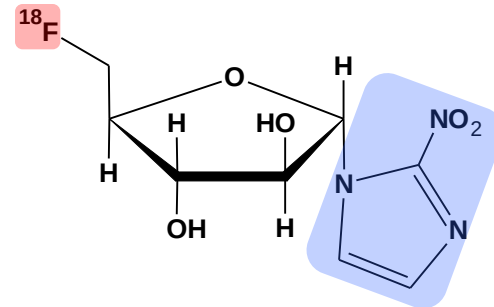


[¹⁸F]FMISO

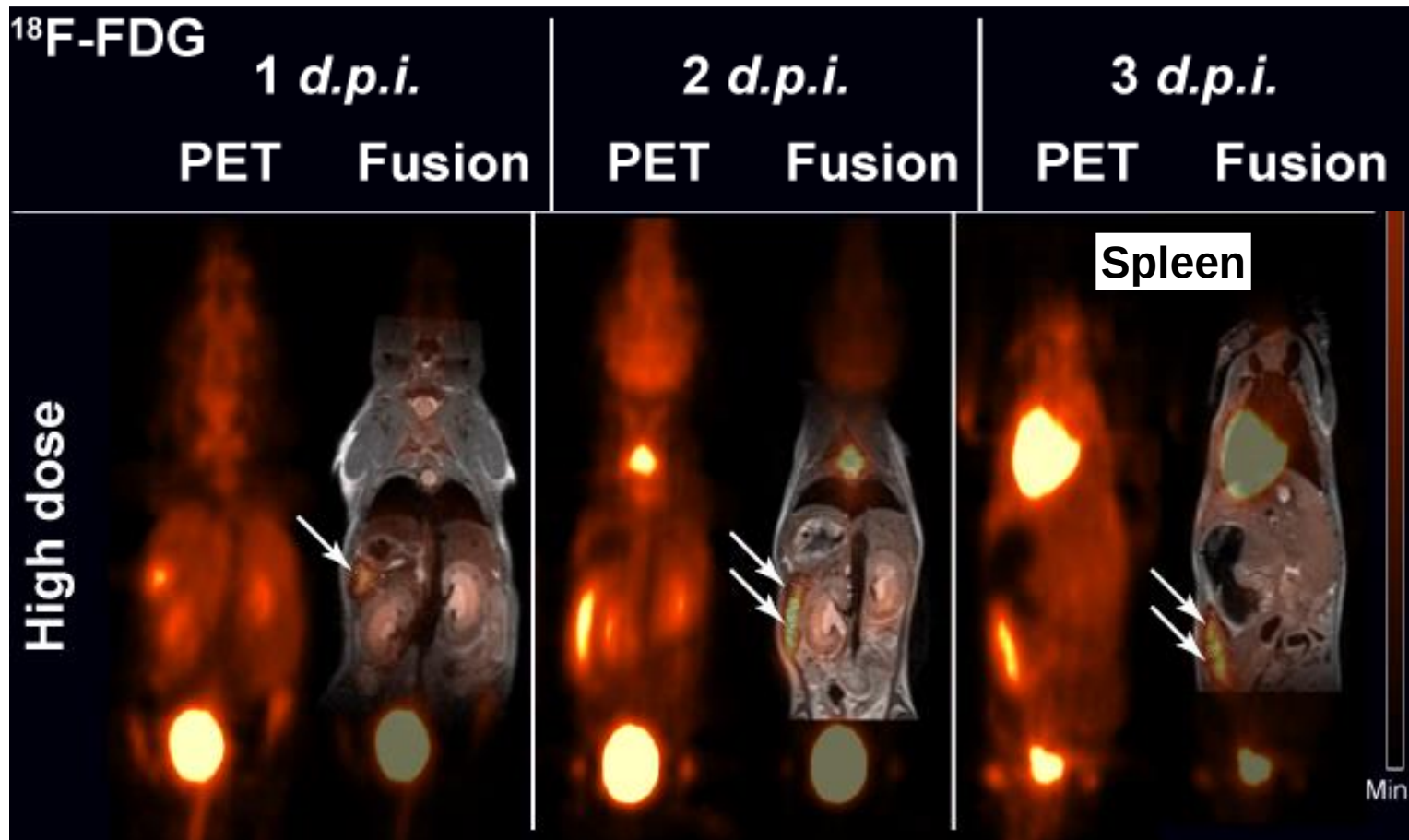


Hypoxia

[¹⁸F]FAZA



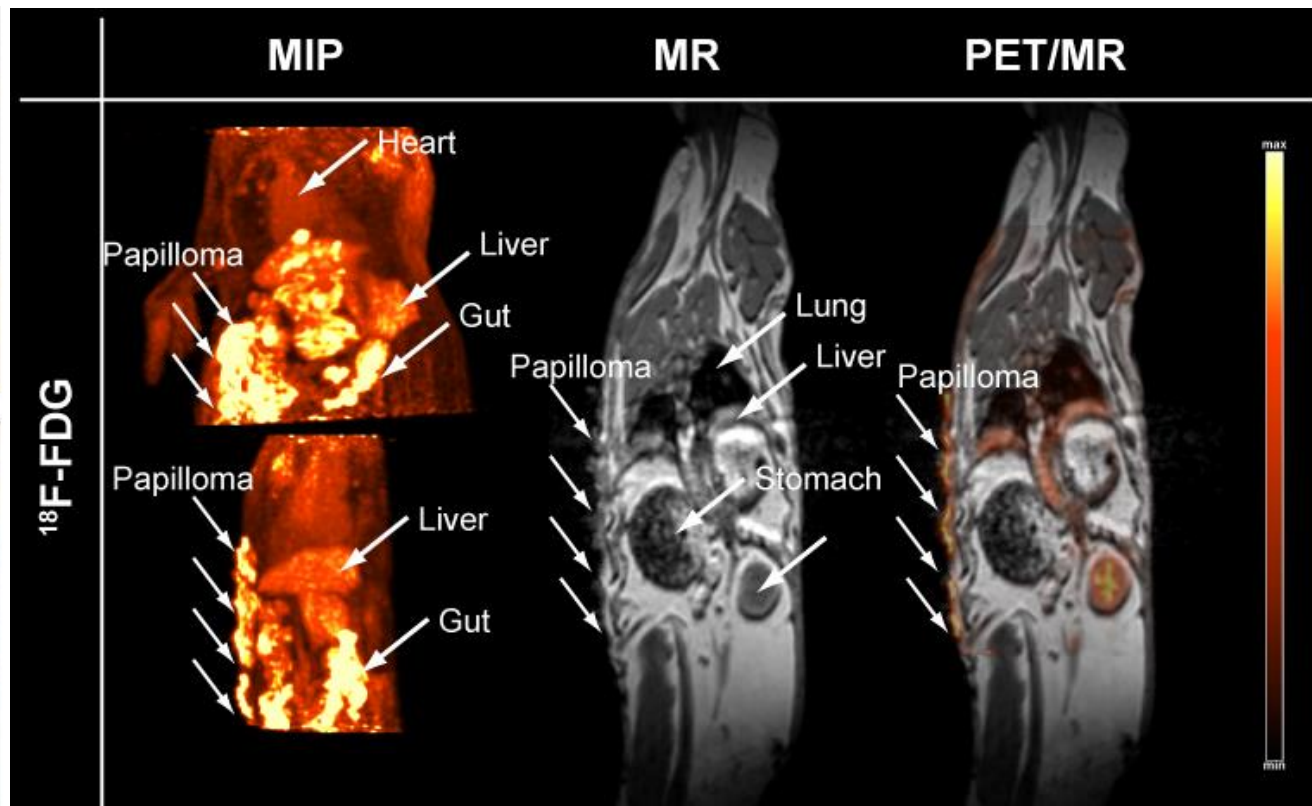
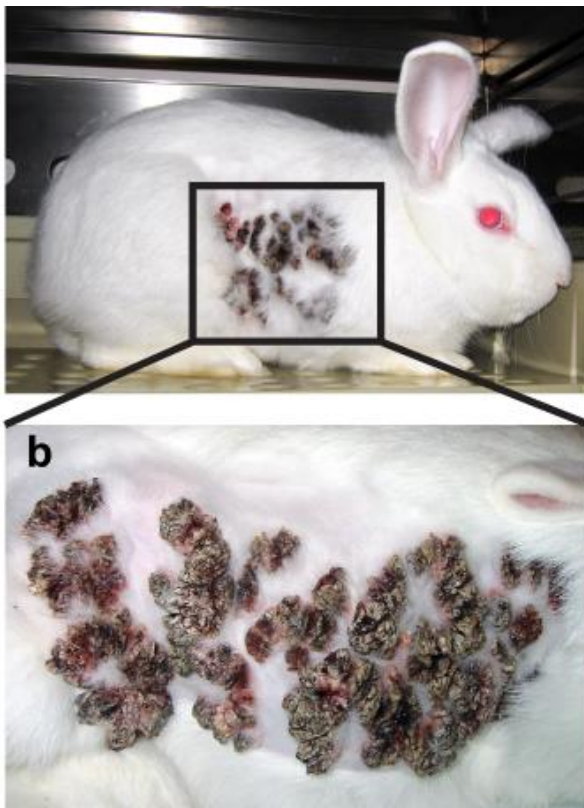
Yersinia enterocolitica bacteria infected mice



unpublished data, Wiehr & Autenrieth

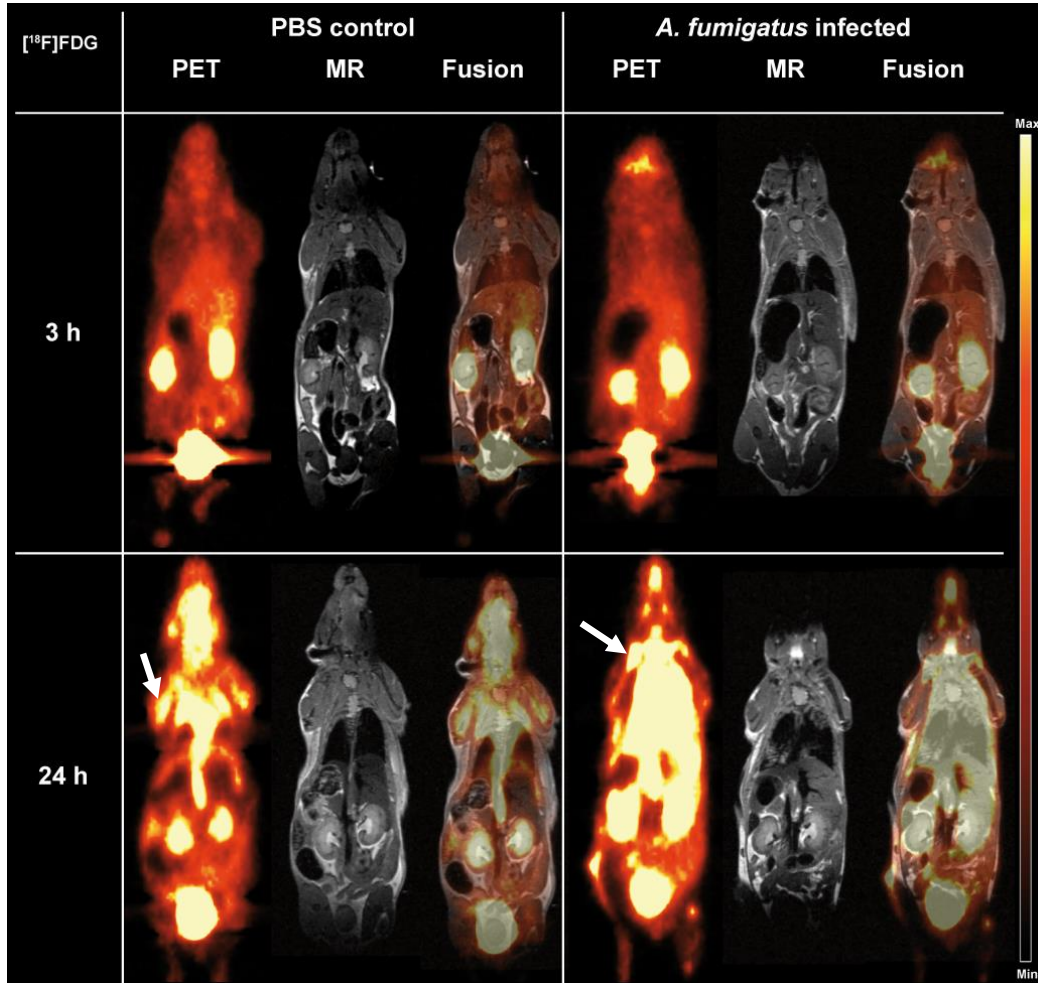


Papilloma virus infected rabbits

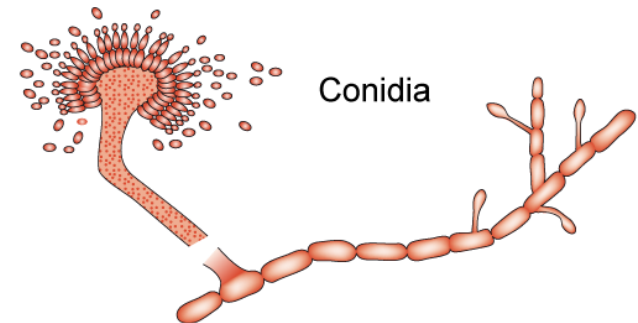


S. Probst & S. Wiehr et al. Molecular Imaging 2014

Fungi *Aspergillus fumigatus* infected mice



Aspergillus fumigatus





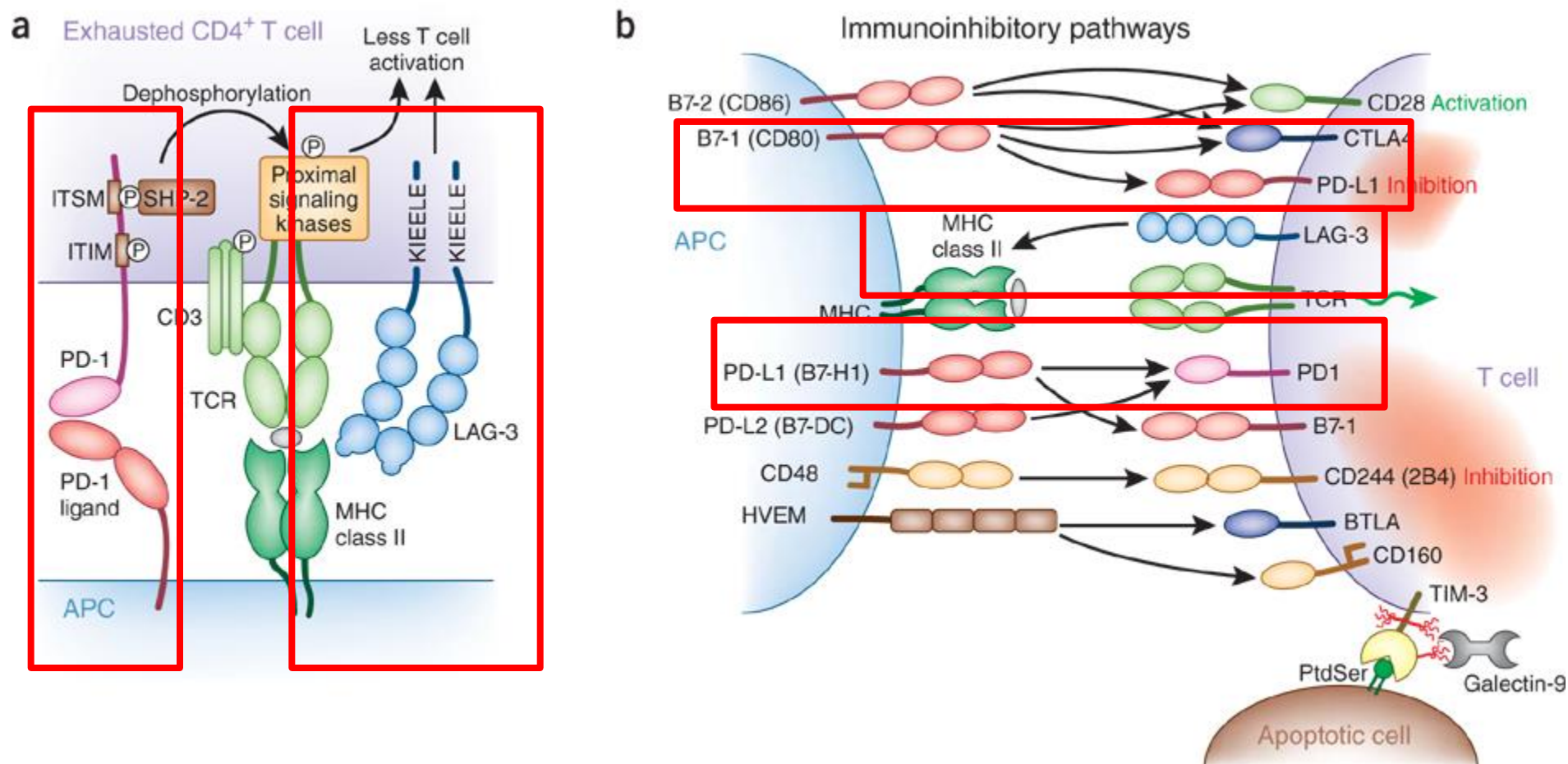
ImmunoPET/MR imaging allows specific detection of *Aspergillus fumigatus* lung infection in vivo

Anna-Maria Rolle^{a,1}, Mike Hasenberg^{b,1}, Christopher R. Thornton^c, Djamschid Solouk-Saran^b, Linda Männ^b, Juliane Weski^b, Andreas Maurer^a, Eliane Fischer^d, Philipp R. Spycher^d, Roger Schibli^d, Frederic Boschetti^e, Sabine Stegemann-Koniszewski^{f,g}, Dunja Bruder^{f,g}, Gregory W. Severin^{h,i}, Stella E. Autenrieth^j, Sven Krappmann^k, Genna Davies^c, Bernd J. Pichler^a, Matthias Gunzer^{b,2}, and Stefan Wiehr^{a,2}

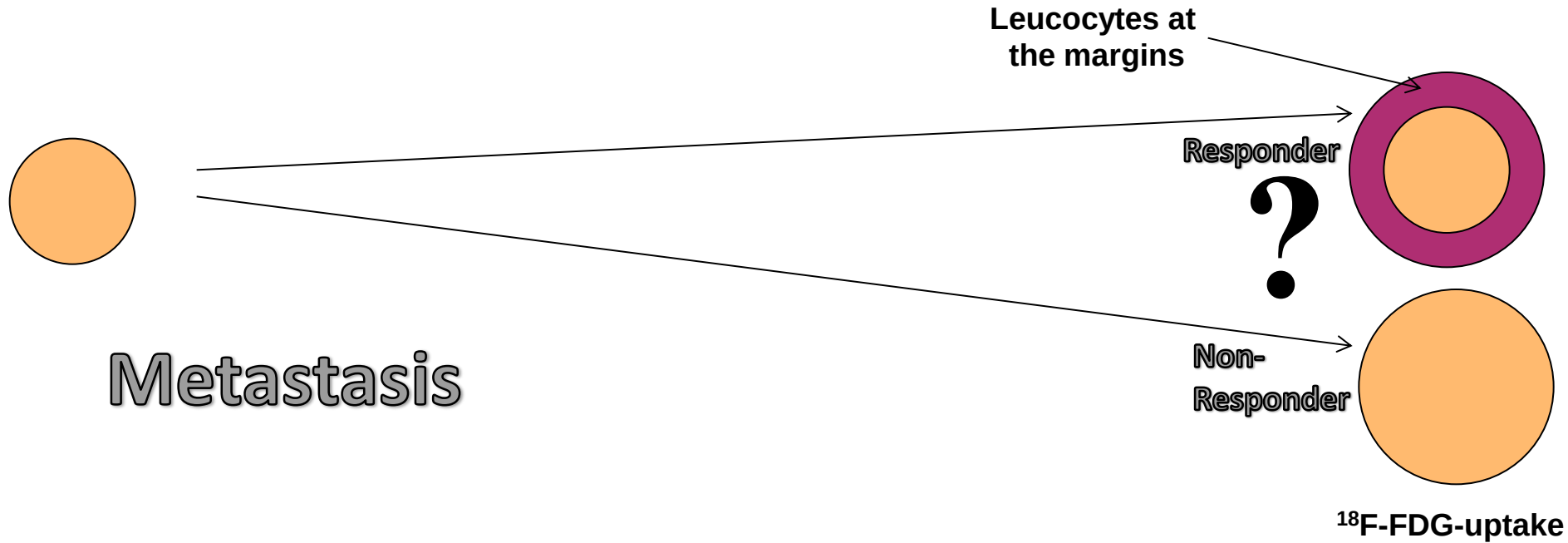
^aWerner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, Eberhard Karls University Tübingen, 72076 Tübingen, Germany;

^bInstitute for Experimental Immunology and Imaging, University Hospital, University Duisburg-Essen, 45122 Essen, Germany; ^cBiosciences and ISCA Diagnostics Ltd., College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom; ^dCenter for Radiopharmaceutical Sciences, Paul Scherrer Institute, 5232 Villigen, Switzerland; ^eCheMatech, Faculté des Sciences Mirande, 21000 Dijon, France; ^fImmune Regulation Group, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany; ^gInfection Immunology, Institute of Medical Microbiology, Infection Control and Prevention, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany; ^hThe Hevesy Laboratory, Center for Nuclear Technologies, Technical University of Denmark, 4000 Roskilde, Denmark; ⁱCenter for Nanomedicine and Theranostics, Technical University of Denmark, 2800 Lyngby, Denmark; ^jDepartment of Hematology, Oncology, Rheumatology, Immunology and Pulmonology, University Hospital Tübingen, 72076 Tübingen, Germany; and ^kMicrobiology Institute - Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nürnberg, 91054 Erlangen, Germany

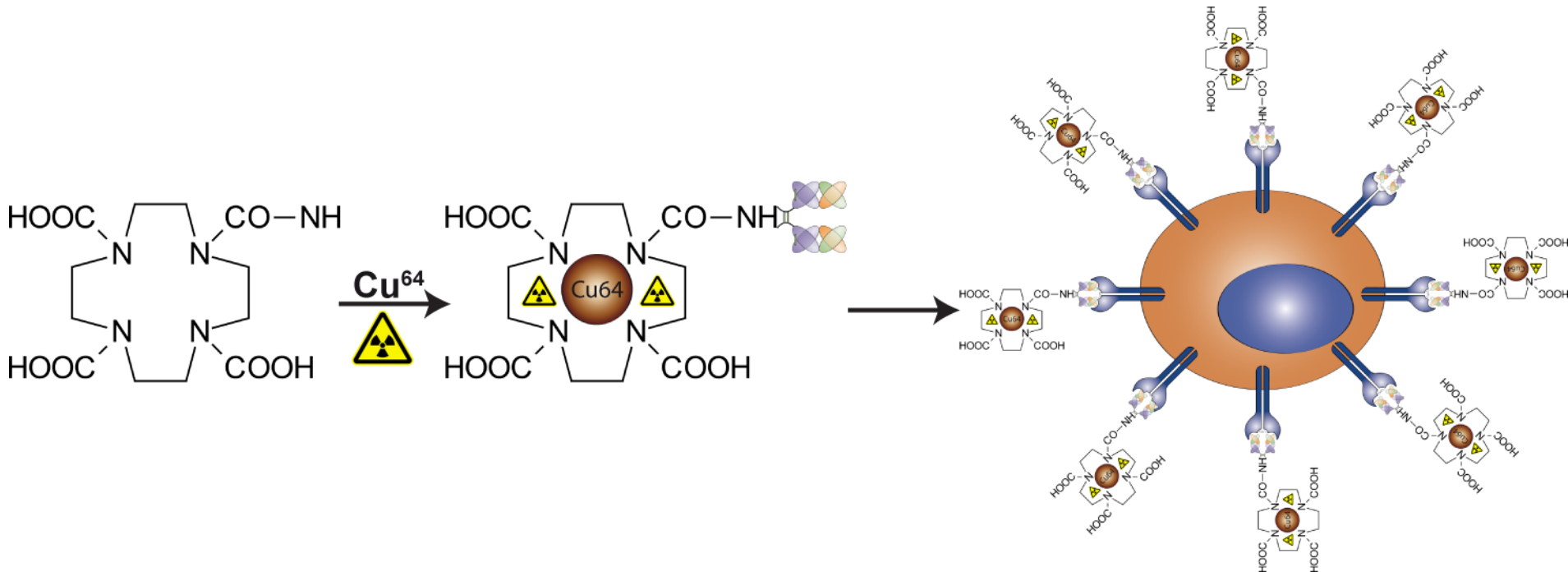
Edited by Michael E. Phelps, University of California, Los Angeles, CA, and approved December 15, 2015 (received for review September 23, 2015)



Gordon J Freeman & Arlene H Sharpe *Nature Immunology* **13**, 113–115 (2012)

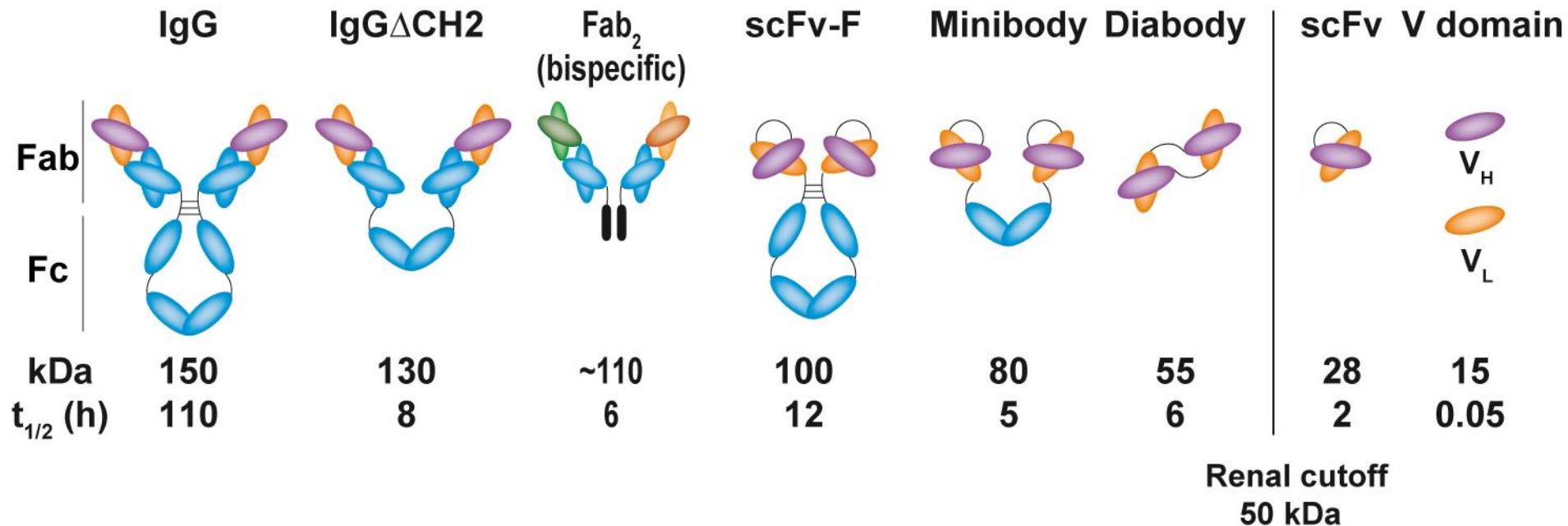


- **During infections** (virus, bacteria, helminthes) PET tracers such as **[¹⁸F]FDG ([¹⁸F]FLT, [¹⁸F]FAZA)** and **Optical Imaging agents** detected only the secondary induced inflammation (“Indirect Detection”)
- The pathogens (virus, bacteria, helminthes) get not visualized.
- Visualization of checkpoint antibody-induced T cell activation



- ***In vivo* radiolabeling of cells (T cells, tumor cells...) or pathogens**

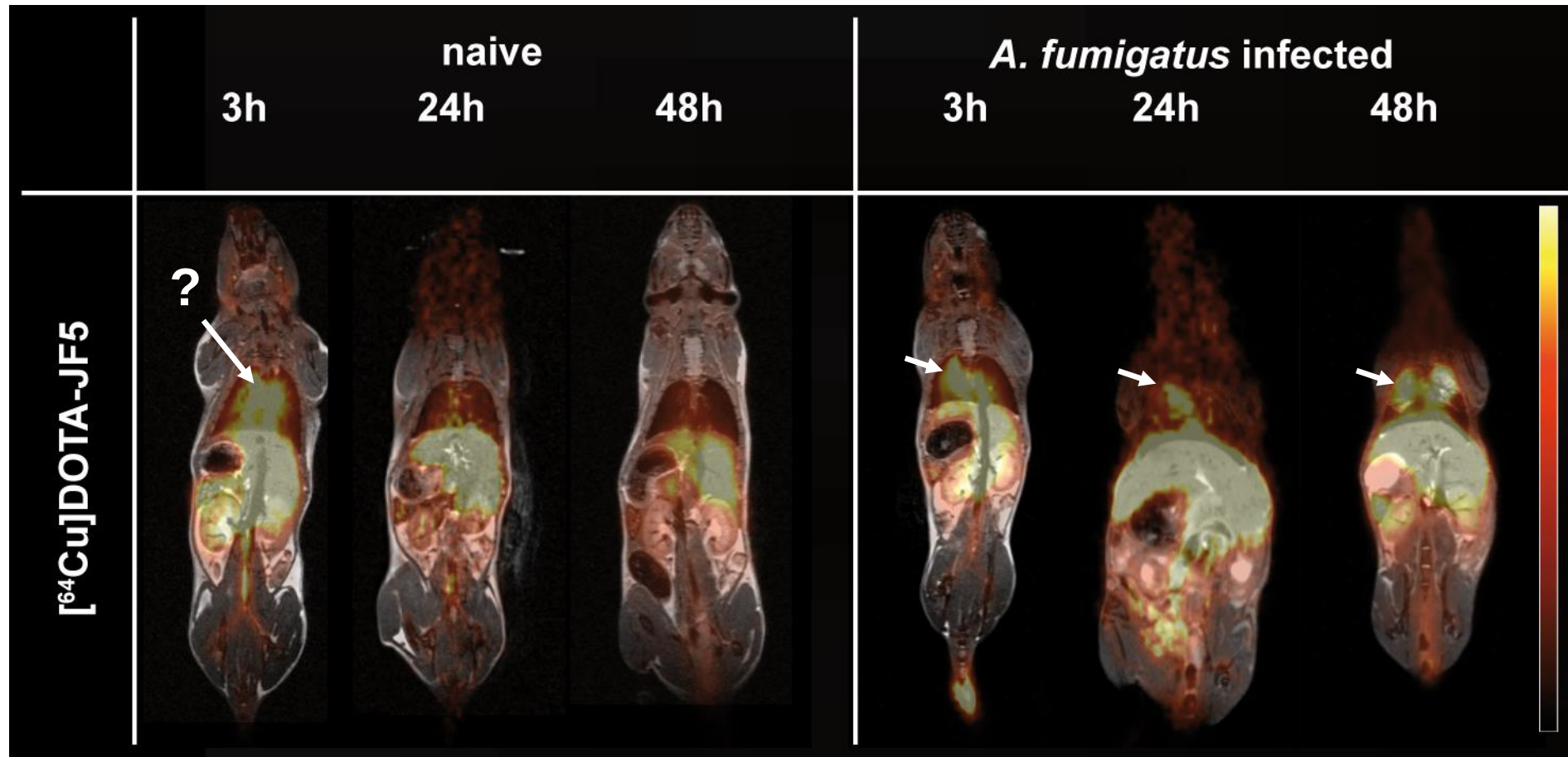
Radiolabeling strategies mAb



modified from Holliger P, Nat Biotechnol. 2005



Aspergillus fumigatus



Rolle, Hasenberg, Gunzer, Wiehr & Pichler



- During infections (virus, bacteria, helminthes) intracellular [^{64}Cu]PTSM-labeling as well as extracellular [^{64}Cu]DOTA-Ab-labeling enables visualization of the pathogens or of cells of interest.



ImmunoPET/MR imaging allows specific detection of *Aspergillus fumigatus* lung infection in vivo

Anna-Maria Rolle^{a,1}, Mike Hasenberg^{b,1}, Christopher R. Thornton^c, Djamschid Solouk-Saran^b, Linda Männ^b, Juliane Weski^b, Andreas Maurer^a, Eliane Fischer^d, Philipp R. Spycher^d, Roger Schibli^d, Frederic Boschetti^e, Sabine Stegemann-Koniszewski^{f,g}, Dunja Bruder^{f,g}, Gregory W. Severin^{h,i}, Stella E. Autenrieth^j, Sven Krappmann^k, Genna Davies^c, Bernd J. Pichler^a, Matthias Gunzer^{b,2}, and Stefan Wiehr^{a,2}

^aWerner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, Eberhard Karls University Tübingen, 72076 Tübingen, Germany;

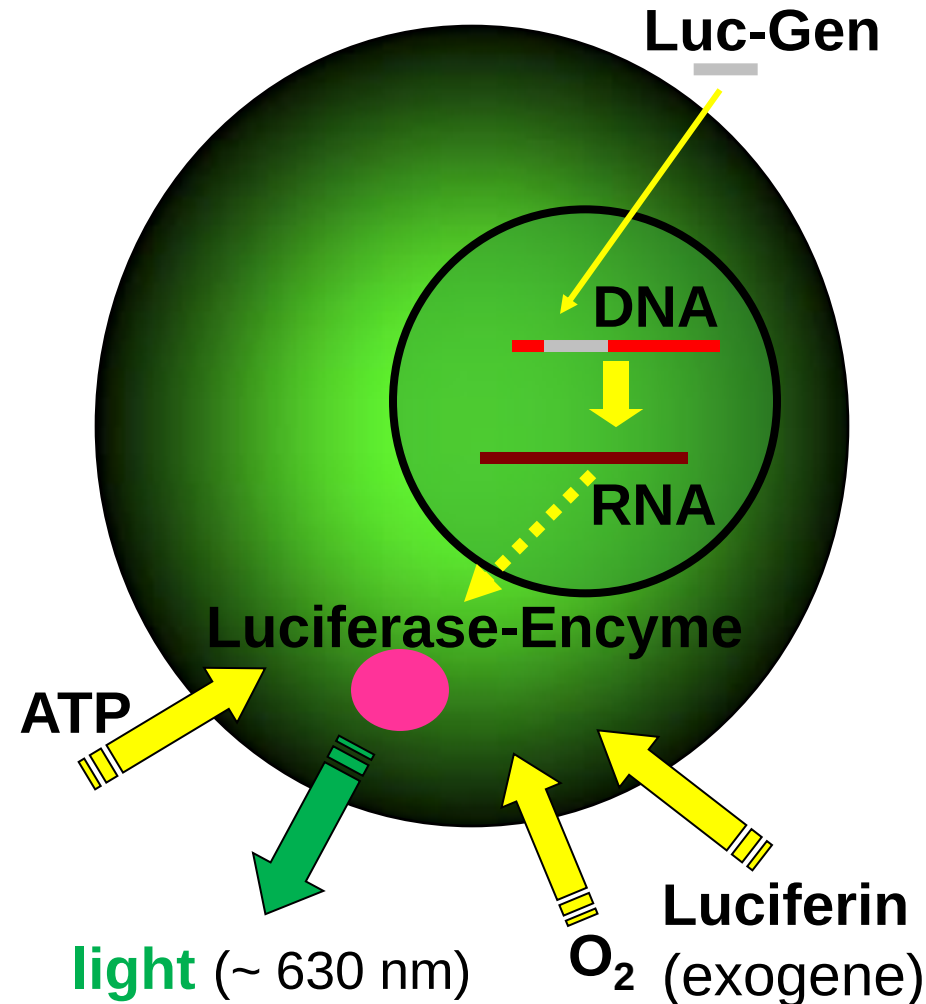
^bInstitute for Experimental Immunology and Imaging, University Hospital, University Duisburg-Essen, 45122 Essen, Germany; ^cBiosciences and ISCA Diagnostics Ltd., College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, United Kingdom; ^dCenter for Radiopharmaceutical Sciences, Paul Scherrer Institute, 5232 Villigen, Switzerland; ^eCheMatech, Faculté des Sciences Mirande, 21000 Dijon, France; ^fImmune Regulation Group, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany; ^gInfection Immunology, Institute of Medical Microbiology, Infection Control and Prevention, Otto-von-Guericke University Magdeburg, 39120 Magdeburg, Germany; ^hThe Hevesy Laboratory, Center for Nuclear Technologies, Technical University of Denmark, 4000 Roskilde, Denmark; ⁱCenter for Nanomedicine and Theranostics, Technical University of Denmark, 2800 Lyngby, Denmark; ^jDepartment of Hematology, Oncology, Rheumatology, Immunology and Pulmonology, University Hospital Tübingen, 72076 Tübingen, Germany; and ^kMicrobiology Institute - Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen and Friedrich-Alexander University Erlangen-Nürnberg, 91054 Erlangen, Germany

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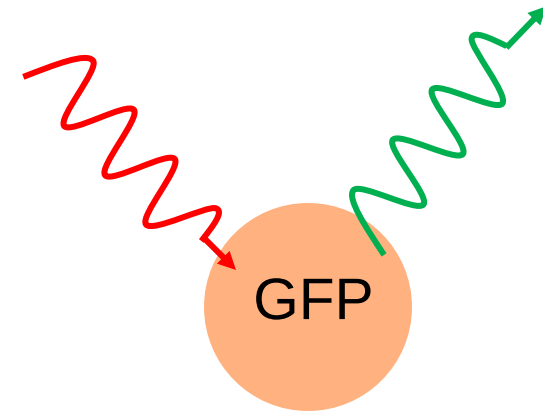
Bioluminescence



Fluorescence

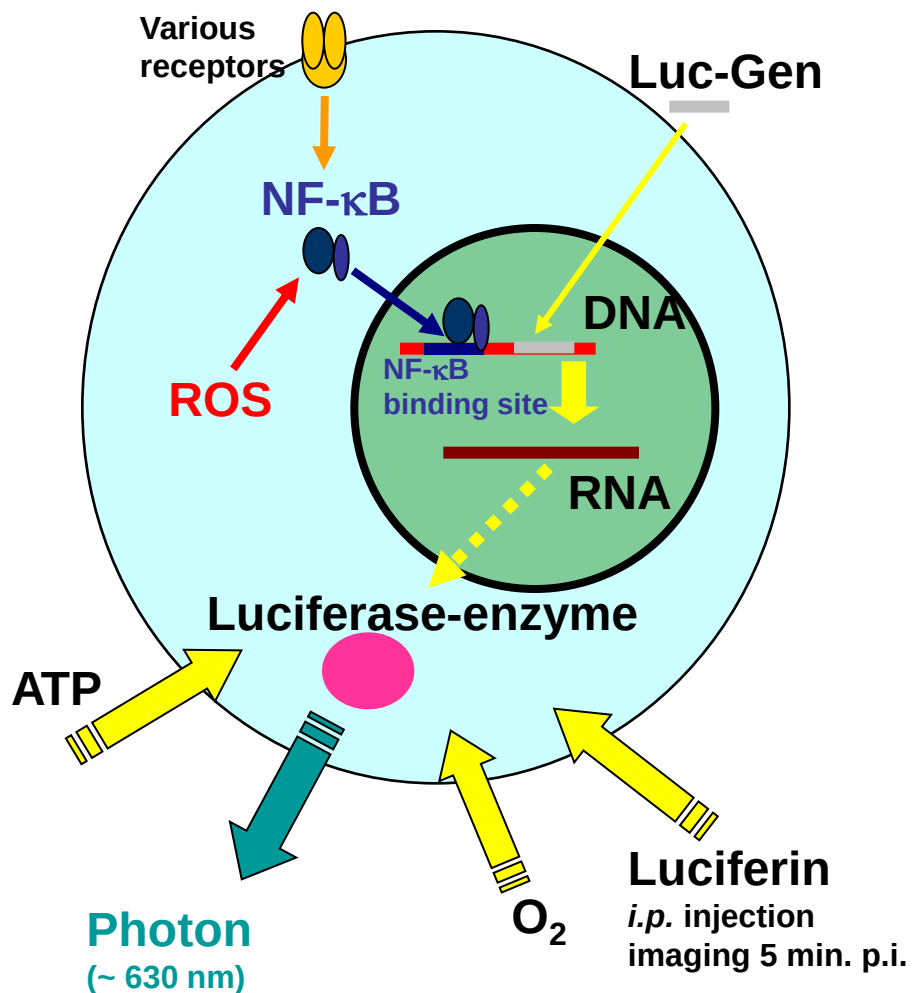
Excitation
~480 nm

Emission
~520 nm

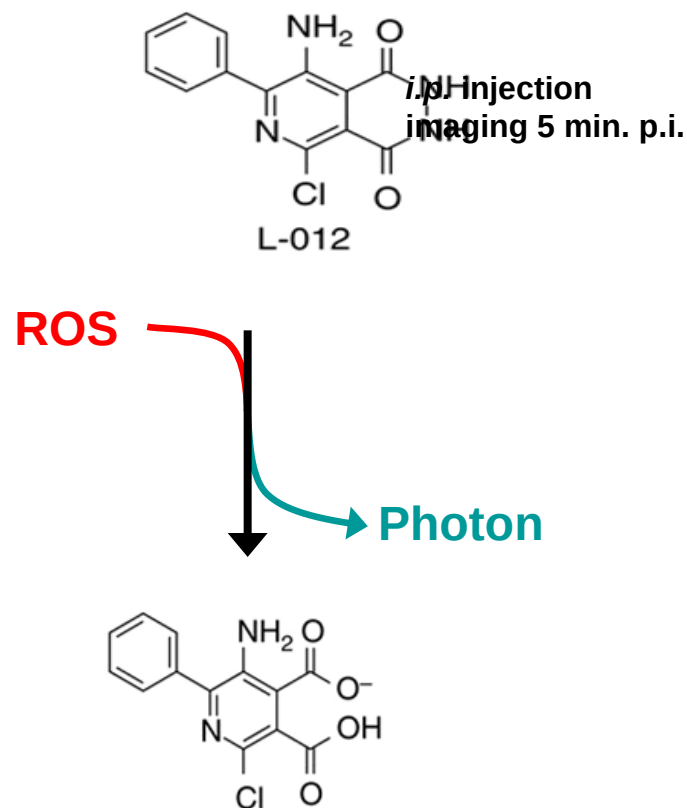


An external **Excitation Light** excites the fluorophore to a higher energy state, followed by **Emission** of photons at a longer wavelength.

Bioluminescence: NF- κ B reporter mice



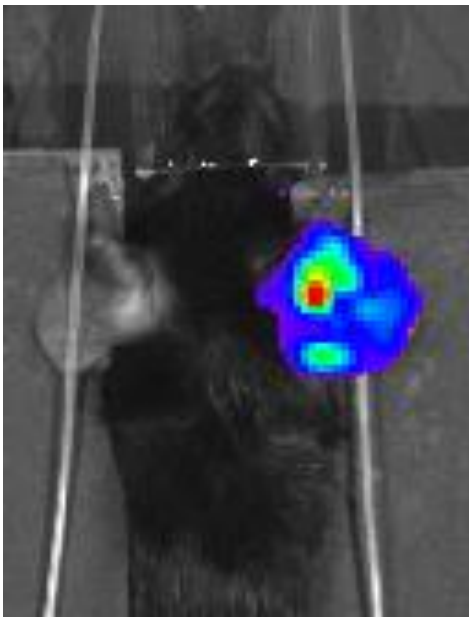
Chemiluminescence: L-012



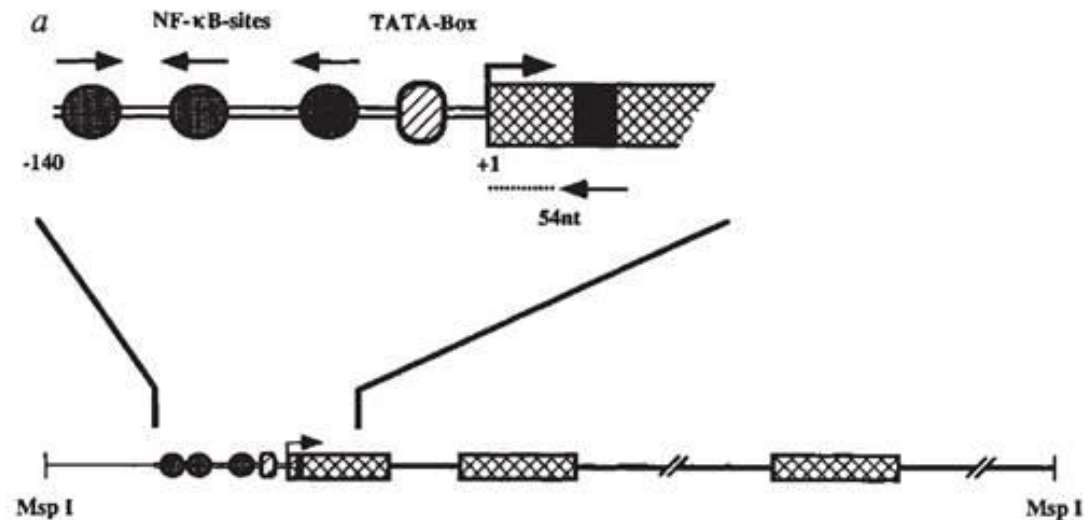
Goiffon et al. Nat. Commun (2015)



NF- κ B: NF- κ B luciferase mice contain three NF- κ B sites from the Ig light chain promoter coupled to the gene encoding firefly luciferase



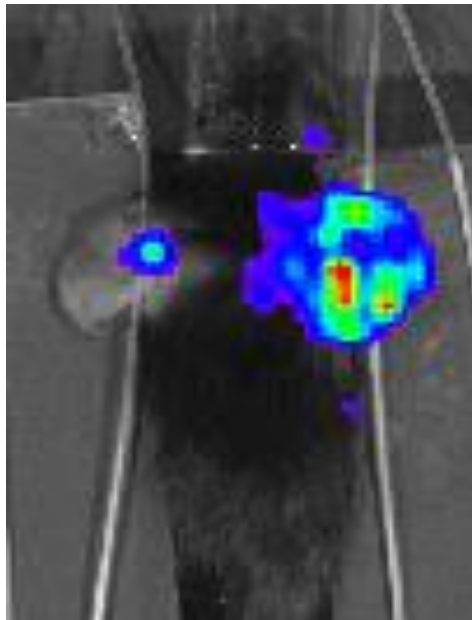
NF- κ B Luc



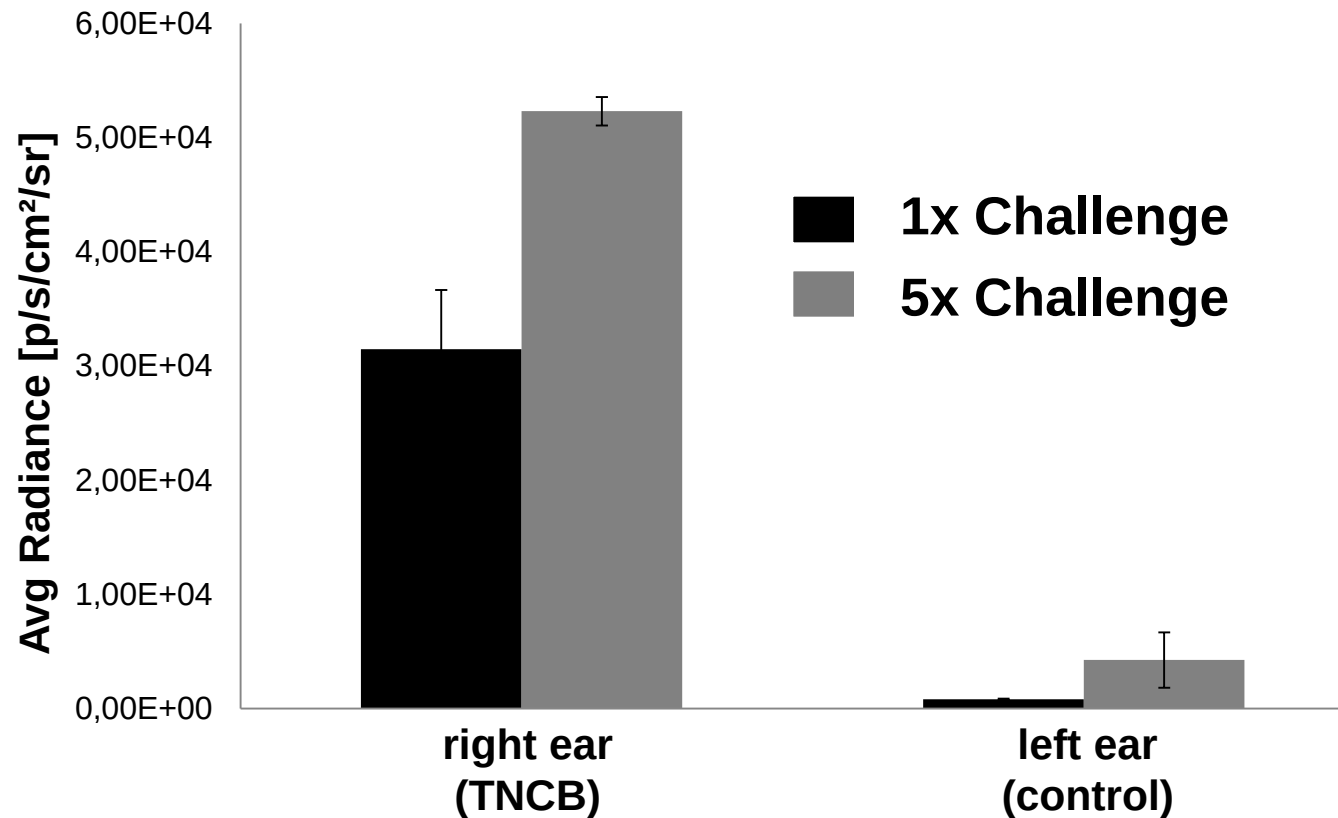
Carlsen H et al., J Immunol. 2002

Reactive Oxygen Species (ROS):

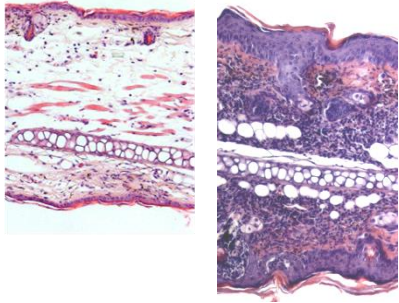
L-012 (8-amino-5-chloro-7-phenylpyrido[3,4-*d*]-pyridazine-1,4(2*H*,3*H*)dione) reacts with hydroxyl radical ($\text{HO}^\cdot$) and hypochlorous acid (HOCl) = Chemiluminescence



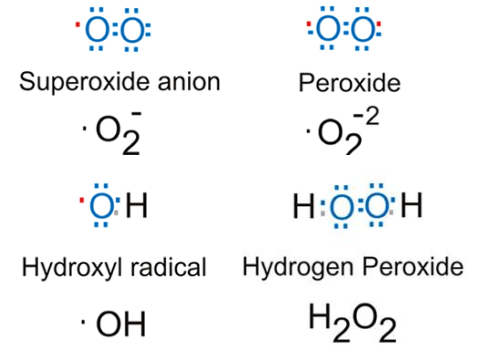
L-012



acute – chronic
DTHR



Neutrophils
→ Macrophages → **ROS-stress**
i.e.



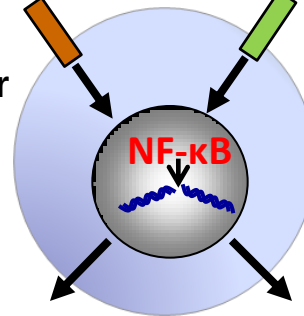
+ **-**
NF-κB
activation

Canonical
p50

- TNF receptor
- IL-1 receptor
- Toll-like receptor
- B-cell receptor
- ...

Non-canonical
p52

- Lymphotoxin receptor
- BAFF receptor
- Receptor activator of NF-κB
- CD40
- ...



TNF, IL-1 β , IL-6...

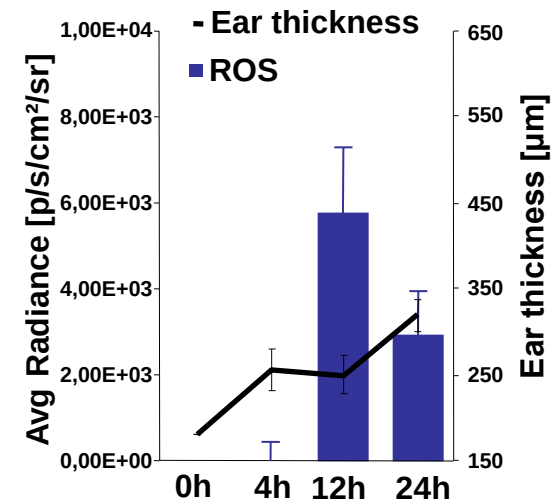
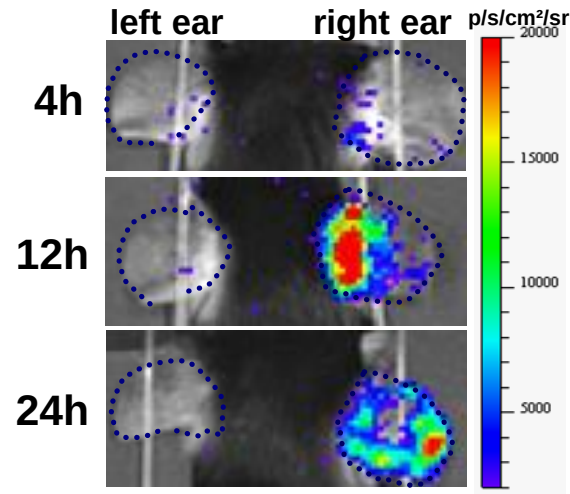
I-TAC (CXCL11), SDF-1 (CXCL12)...



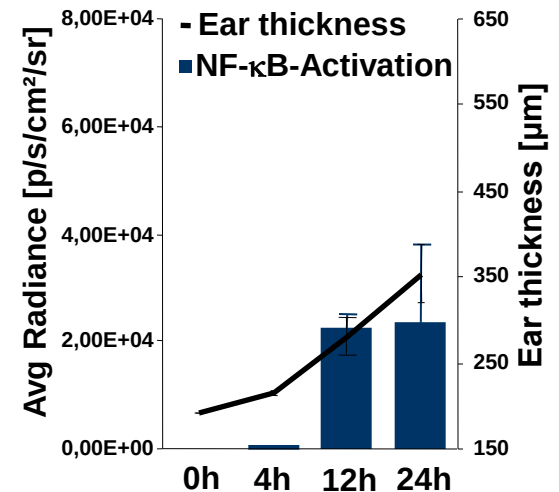
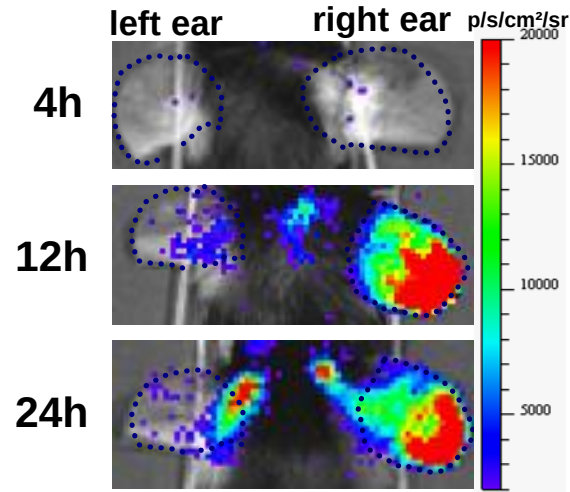
Johannes Schwenck

Results - Acute DTHR 1 x Challenge

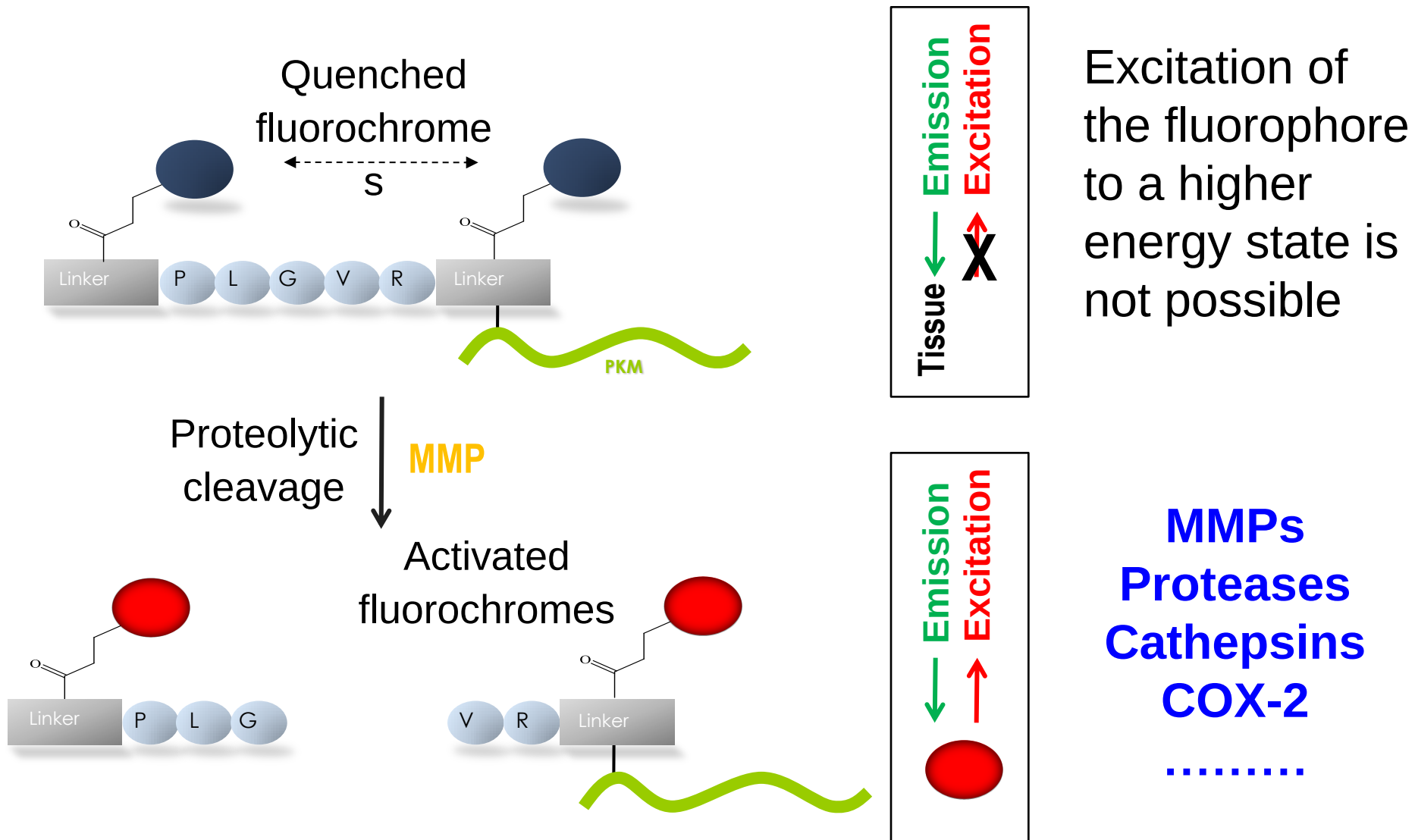
ROS-expression



NF-κB-activity

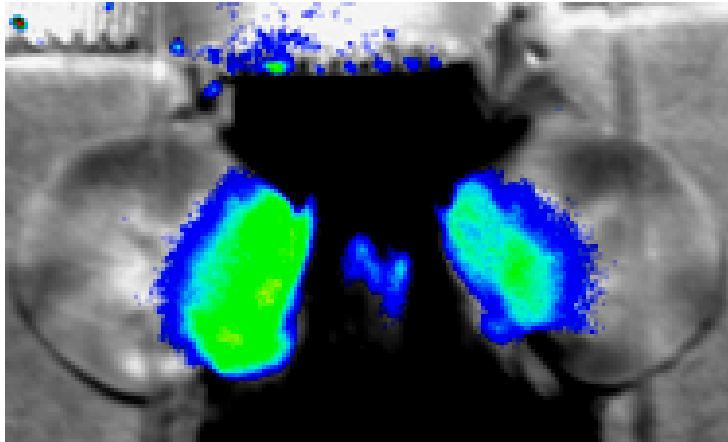


OI - Activatable Fluorescence Probes

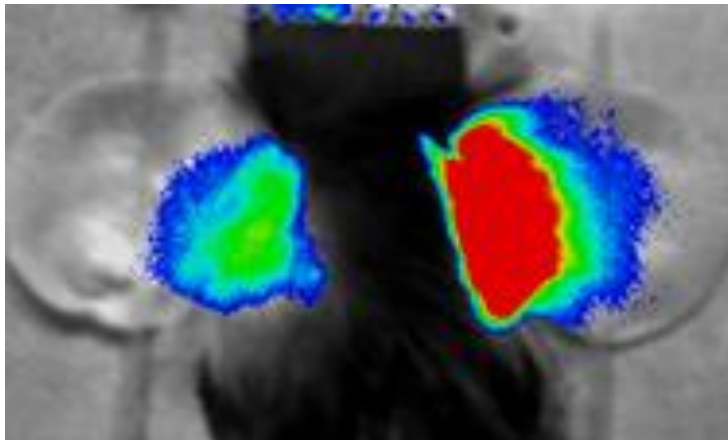




MMPsense680



Control



Acute DTMR



RESEARCH ARTICLE

In Vivo Optical Imaging of Matrix Metalloproteinase Activity Detects Acute and Chronic Contact Hypersensitivity Reactions and Enables Monitoring of the Antiinflammatory Effects of *N*-Acetylcysteine

Johannes Schwenck, Christoph M. Griessinger, Kerstin Fuchs, Daniel Bukala, Nadine Bauer, Martin Eichner, Martin Röcken, Bernd J. Pichler, and Manfred Kneilling

Abstract

The aim of this study was to determine whether the severity of contact hypersensitivity reactions (CHSRs) can be observed by noninvasive in vivo optical imaging of matrix metalloproteinase (MMP) activity and whether this is an appropriate tool for monitoring an antiinflammatory effect. Acute and chronic CHSRs were elicited by application of a 1% trinitrochlorobenzene (TNCB) solution for up to five times on the right ear of TNCB-sensitized mice. *N*-Acetylcysteine (NAC)-treated and sham-treated mice were monitored by measuring ear swelling and optical imaging of MMP activity. In addition, we performed hematoxylin-eosin staining and CD31 immunohistochemistry for histopathologic analysis of the antiinflammatory effects of NAC. The ear thickness and the MMP activity increased in line with the increasing severity of the CHSR. MMP activity was enhanced 2.5- to 2.7-fold during acute CHSR and 3.1- to 4.1-fold during chronic CHSR. NAC suppressed ear swelling and MMP signal intensity in mice with acute and chronic CHSR. During chronic CHSR, the vessel density was significantly reduced in ear sections derived from NAC-treated compared to sham-treated mice. In vivo optical imaging of MMP activity measures acute and chronic CHSR and is useful to monitor antiinflammatory effects.

T CELL-DEPENDENT autoimmune diseases such as rheumatoid arthritis and psoriasis vulgaris are classified as delayed-type hypersensitivity reactions (DTHRs), orchestrated by interferon- γ -producing CD8⁺ (Tc1) and CD4⁺ (Th1) T cells.^{1,2} Most DTHRs, such as cutaneous contact hypersensitivity reactions (CHSRs), are accompanied by accumulations of polymorphonuclear neutrophils (PMNs), which elicit an oxidative burst and are critically involved in the pathogenesis of several autoimmune diseases.³ The oxidative burst is characterized by a rapid release of reactive oxygen species (ROS)

generated by PMNs and macrophages.^{4,5} ROS regulate inflammatory immune responses through interactions with nuclear factor (NF)- κ B or p38 mitogen-activated protein (MAP) kinase signaling pathways⁶ and display either tissue-destructive or -protective effects.⁷

Our previous work focused on studying the effector phase in a murine model of acute and chronic trinitrochlorobenzene (TNCB)-induced CHSR.^{3,8} We observed a strongly reduced PMN accumulation and oxidative burst in inflamed ear tissue of TNFR1^{-/-} and mast cell-deficient mice with acute TNCB-induced CHSR.^{3,8} Using in vivo positron emission tomography (PET) and ¹⁸F-RGD-peptide, which binds to activated $\alpha_v\beta_3$ -integrin, we distinguished acute from chronic TNCB-induced CHSR and followed angiogenesis during the progression of local inflammation.⁹

As chronic inflammation is highly associated with angiogenesis,⁹ antiinflammatory or antiangiogenic drugs can suppress chronic cutaneous DTHR.¹⁰ The underlying mechanisms of inflammation-induced angiogenesis are driven by hypoxia¹¹ and proinflammatory mediators such as tumor necrosis factor (TNF), macrophage inflammatory

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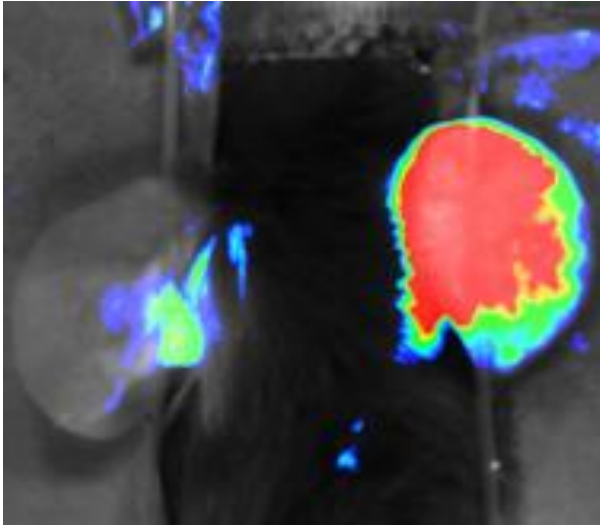
DOI 10.2310/7290.2014.00044

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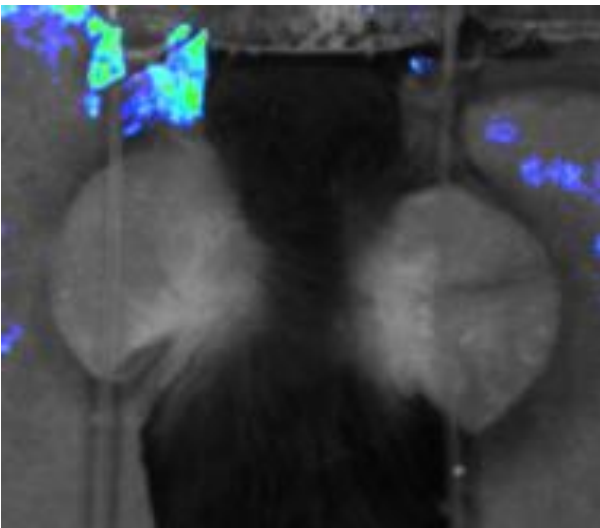
DECKER



Prosense

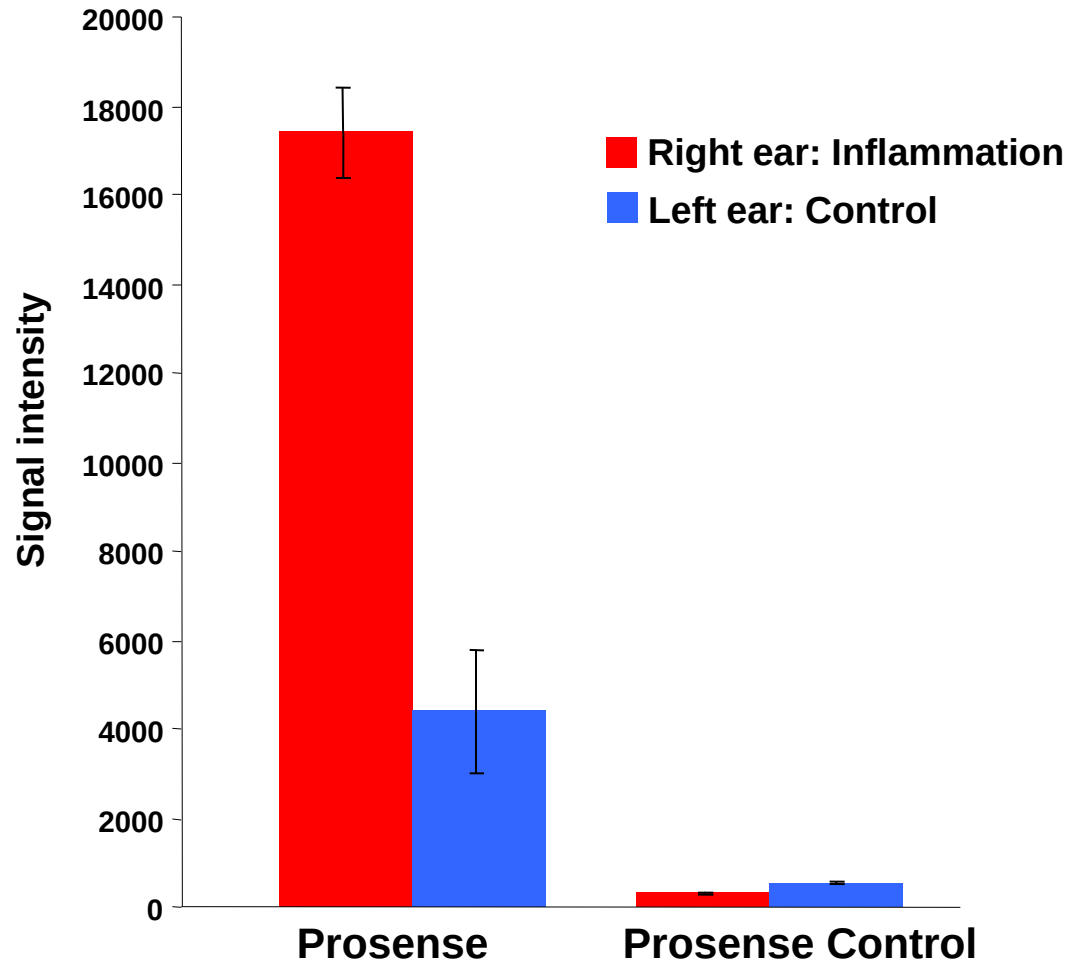


Prosense Control



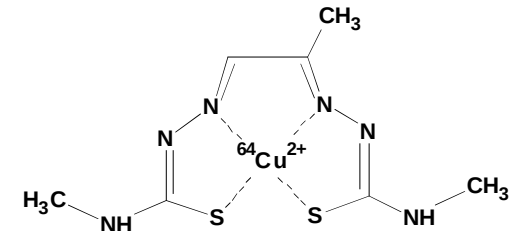
Chronic Cutaneous DTHR

5x Challenge

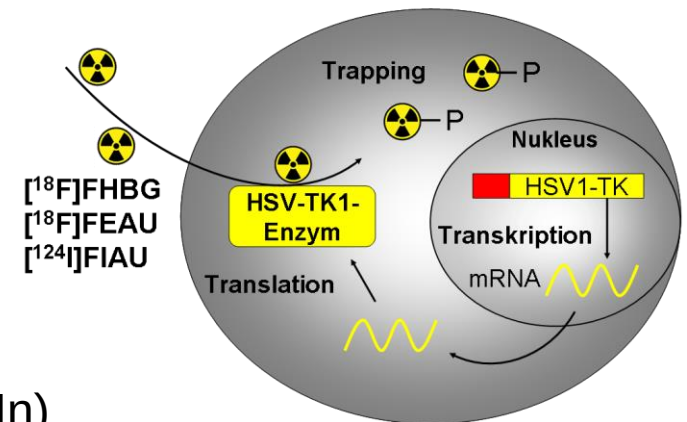




- **Direct labeling**
[^{64}Cu]PTSM, [^{18}F]FDG $\rightarrow$ PET
- **Reportergene**
Herpes Simplex Virus-Thymidin-Kinase (HSV-TK)
- **Transgenic animals**
„mighty mouse“ (Sam Gambhir)
HSV-TK transgenic mouse
- **Labeling cells with antibodies (*ex vivo*)**
via chelator with long living isotopes (^{64}Cu , ^{89}Zr , ^{111}In)

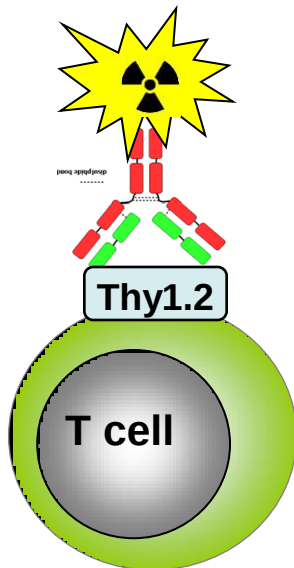


HSV1-TK



Extracellular

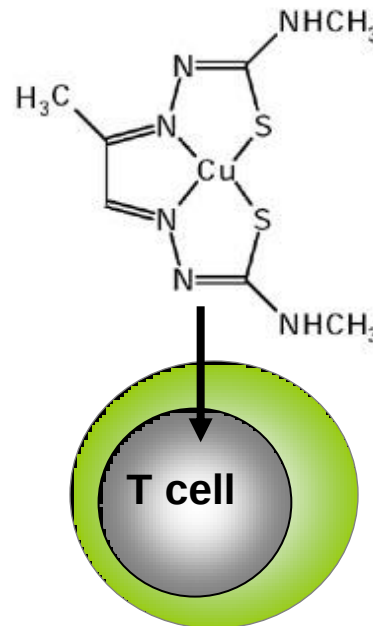
Radiolabeled antibody



Intracellular

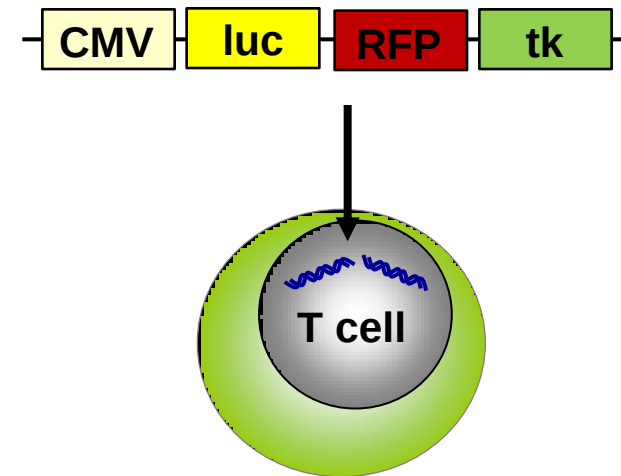
Lipophilic

[⁶⁴Cu]PTSM



Reporter gene

Triple fusion cassette



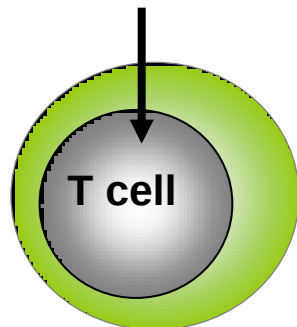
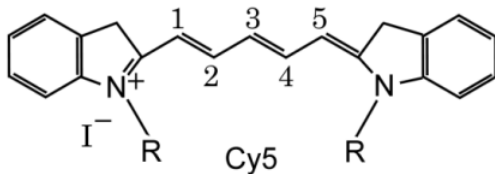


Optical Imaging

Intracellular

Lipophilic

Cyanin Vybrant Solution
Dye (Cy5)

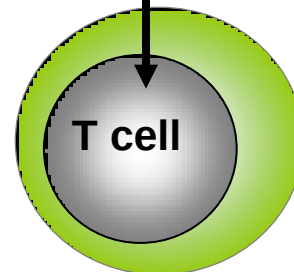
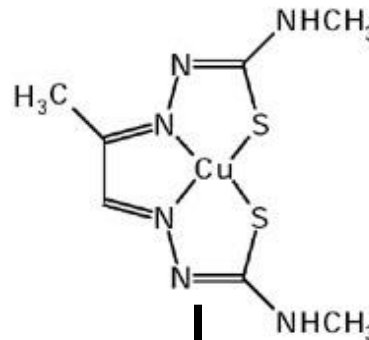


Positron Emission Tomography

Intracellular

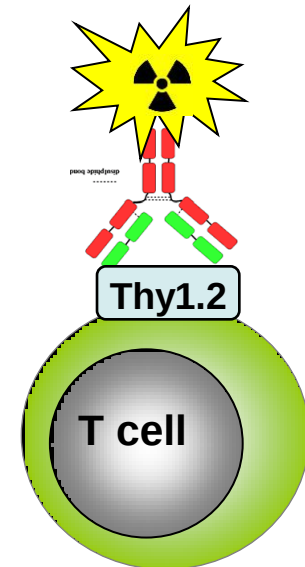
Lipophilic

[⁶⁴Cu]PTSM



Extracellular

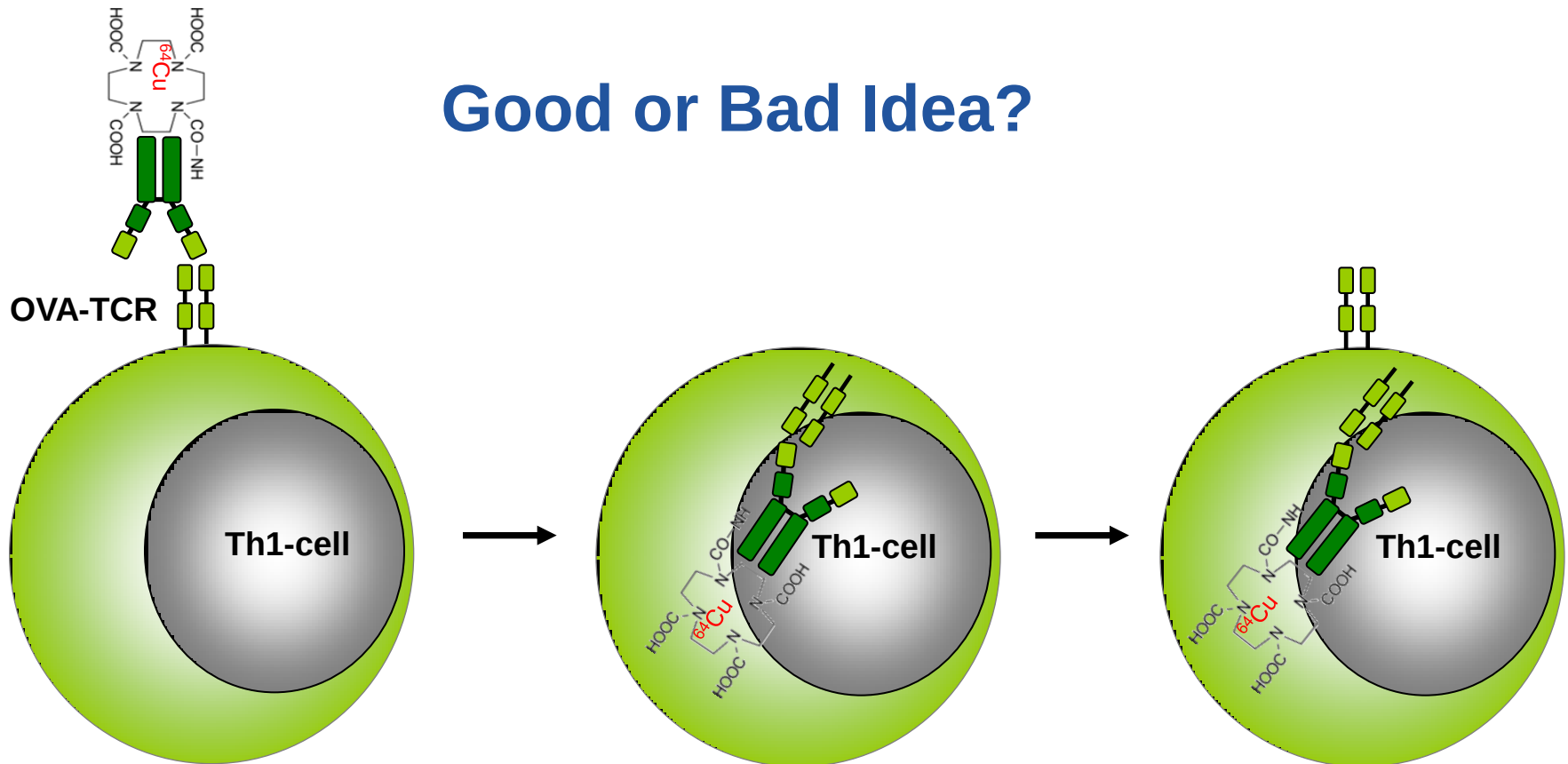
Radiolabeled antibody



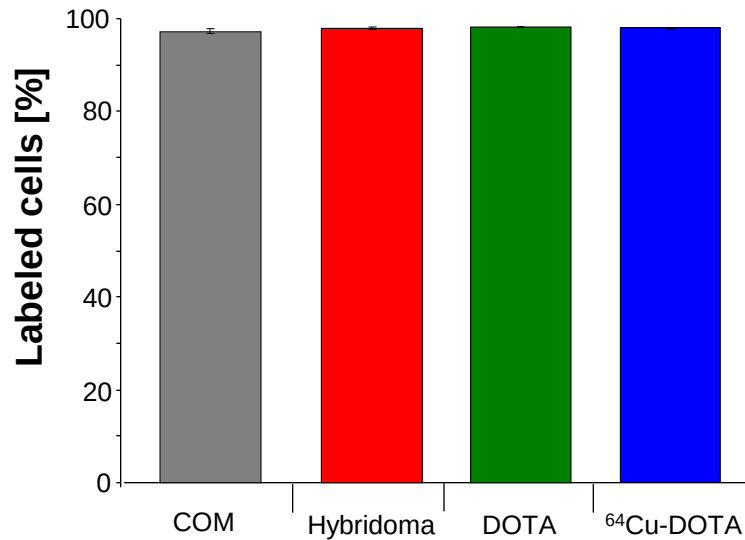


[⁶⁴Cu]DOTA-KJ1-26

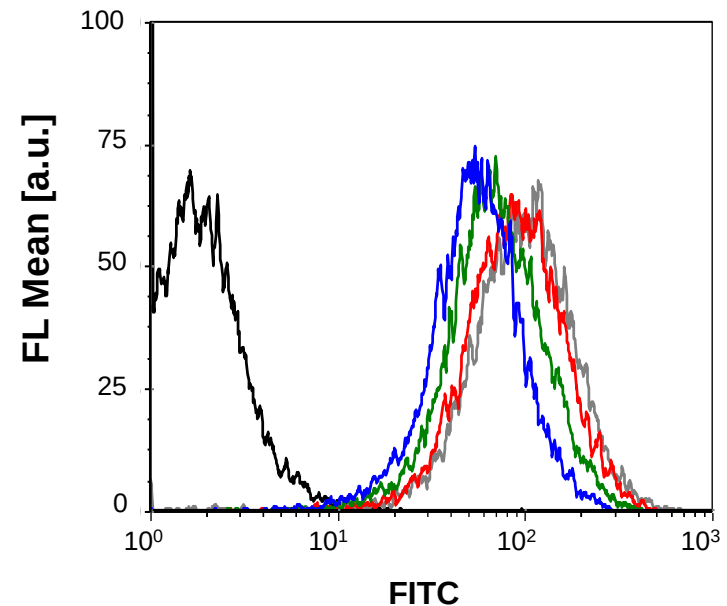
Good or Bad Idea?



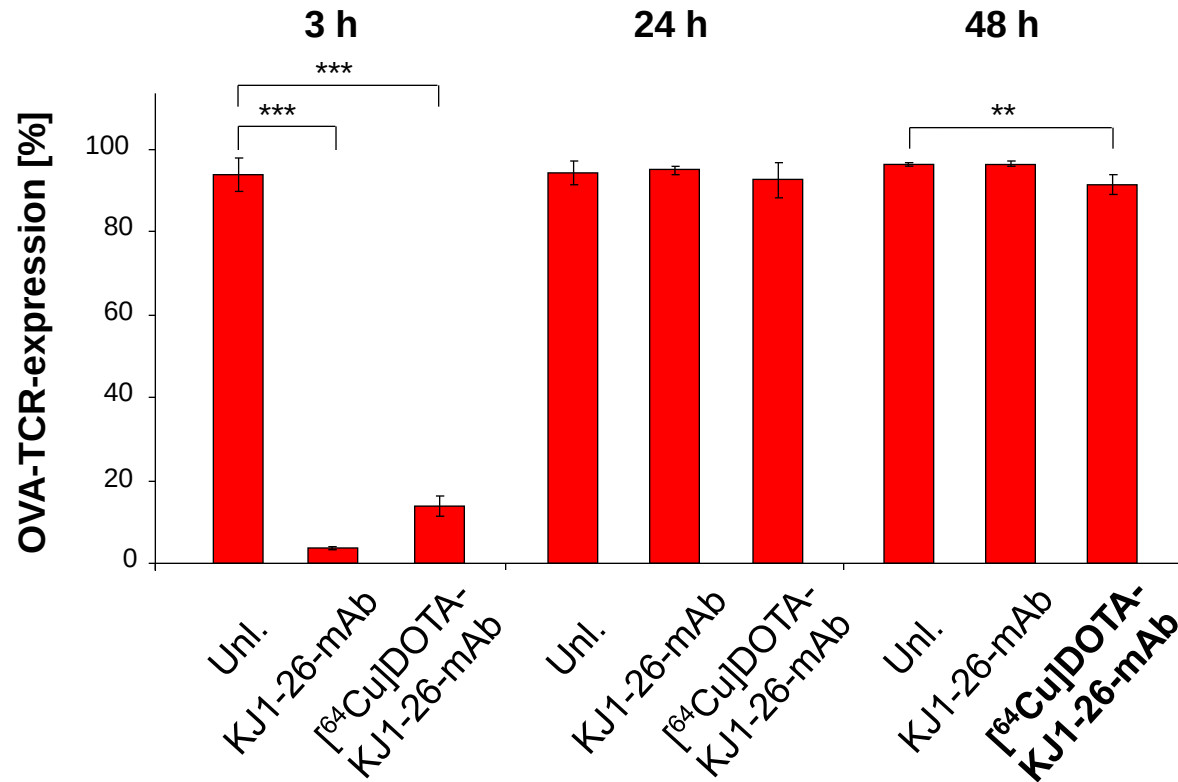
TCR-labelling: Ab against the TCR



KJ1-26-mAb-modification

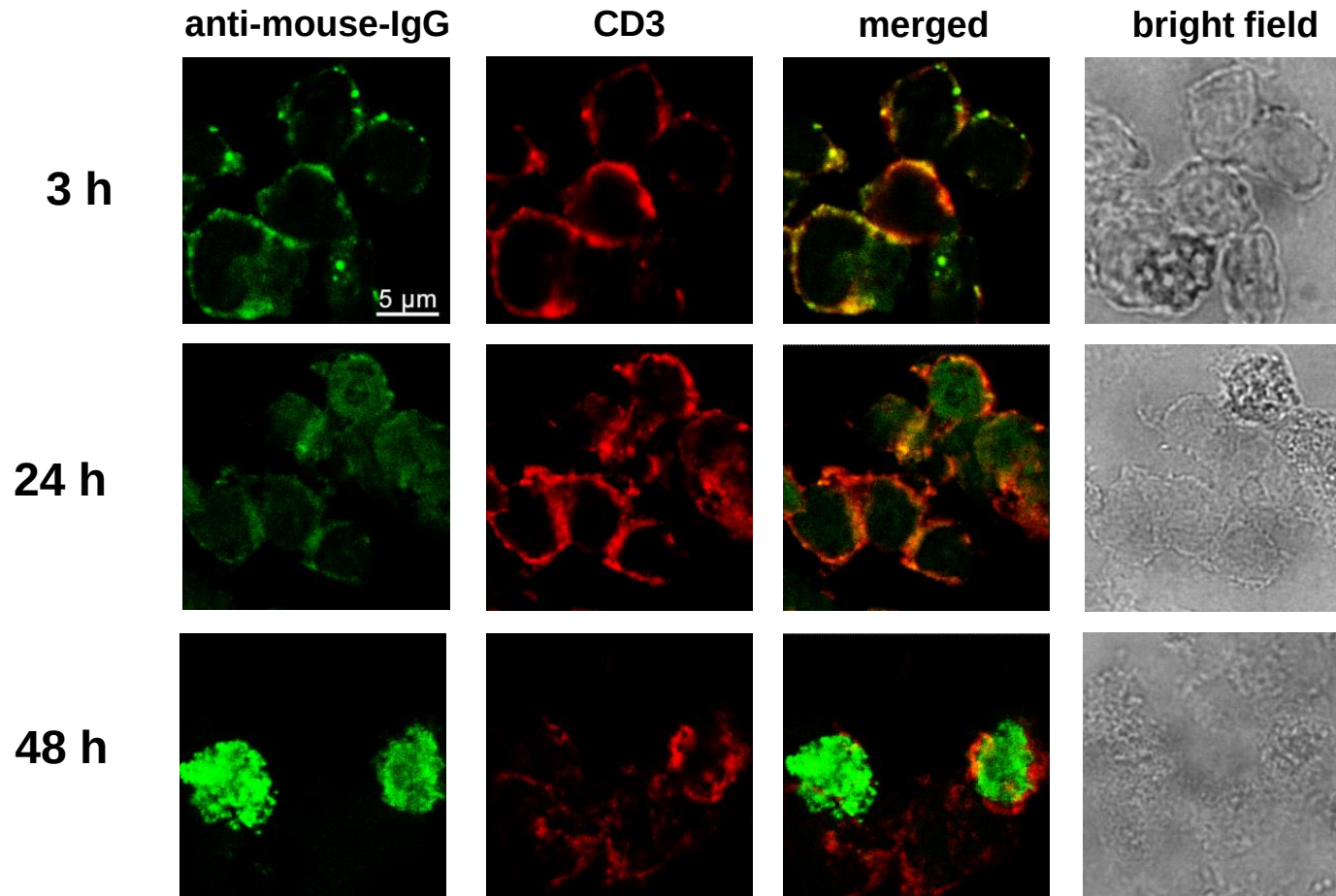


TCR-labelling: Ab against the TCR



TCR-labelling: Ab against the TCR

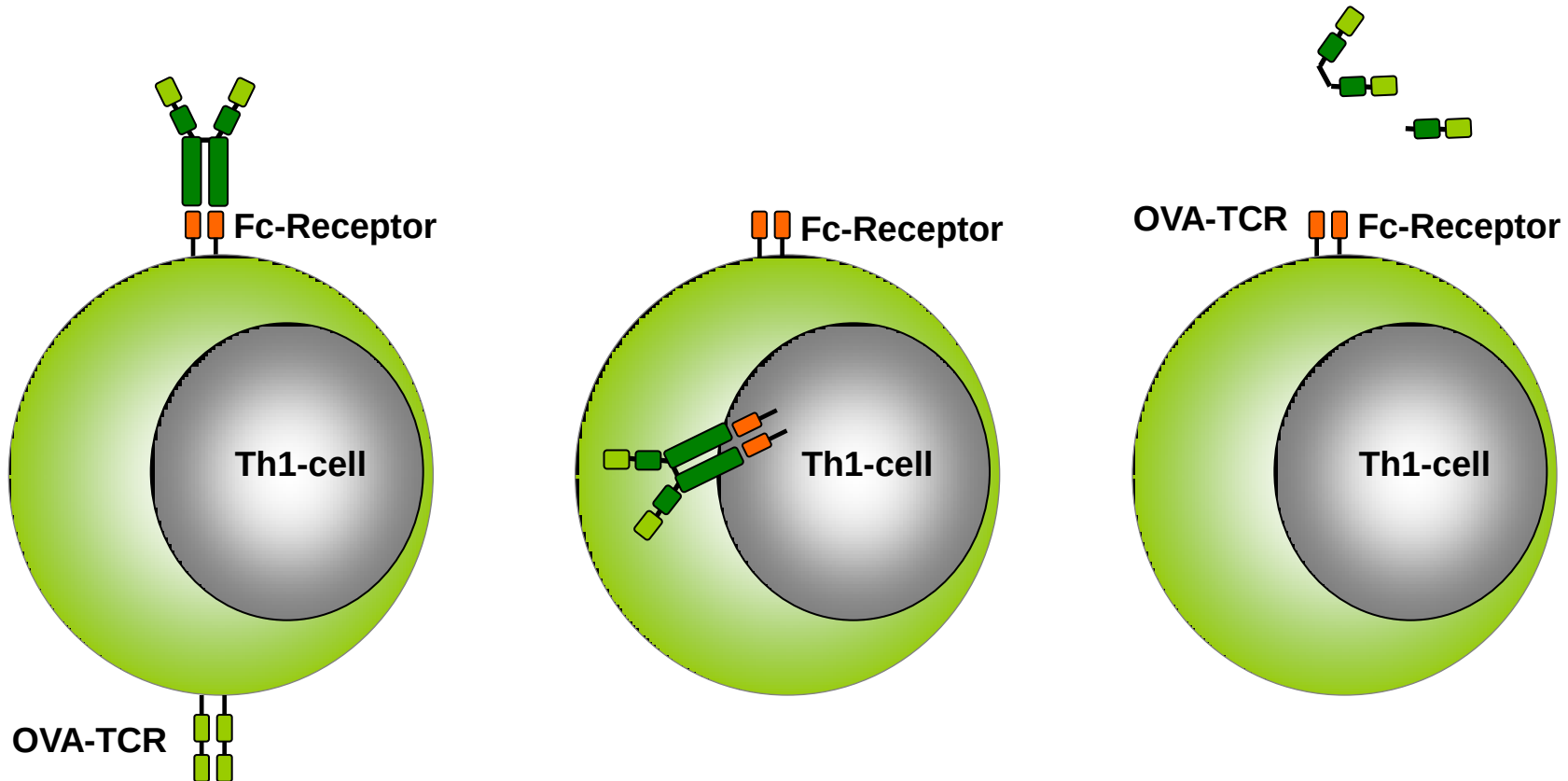
TCR-[⁶⁴Cu]DOTA-KJ1-26-mAb-complex-labeled
cOVA-TCRtg-Th1 cells



TCR-labelling: Ab against the TCR

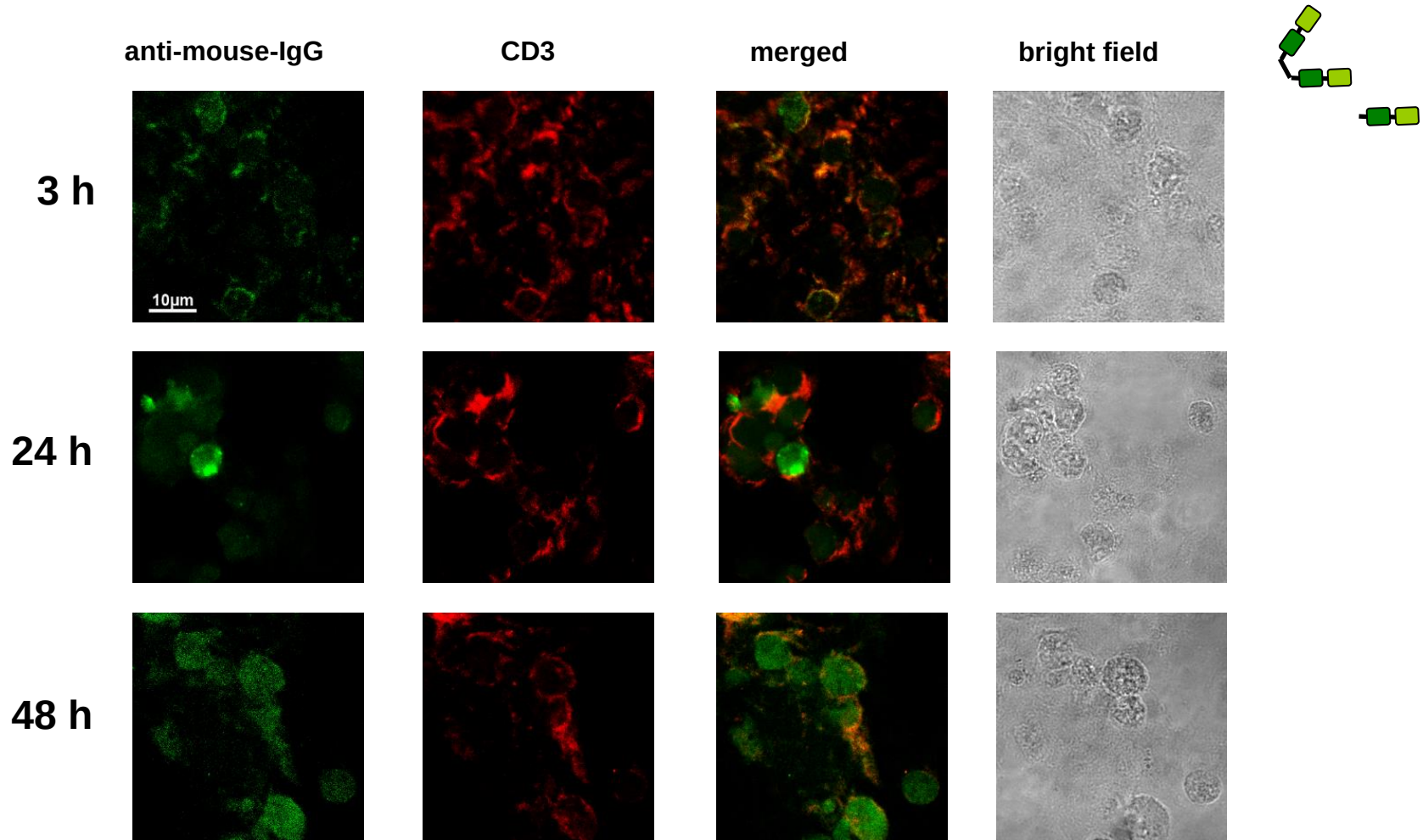


OVA-F(ab)/F(ab)₂-Fragments

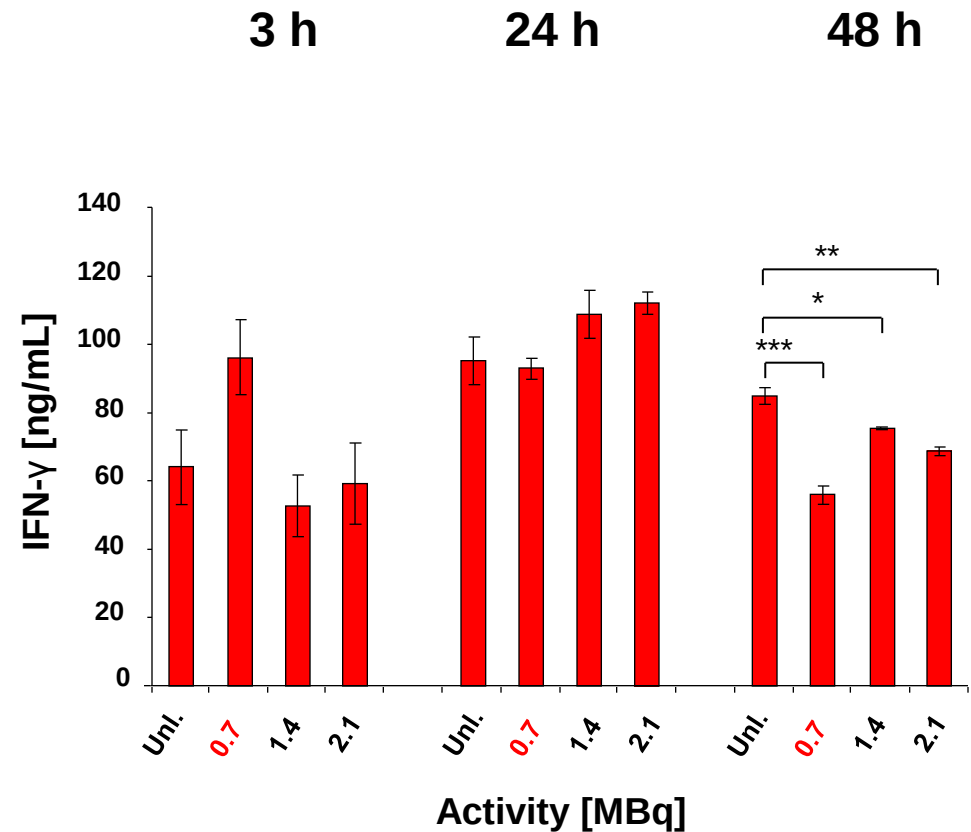
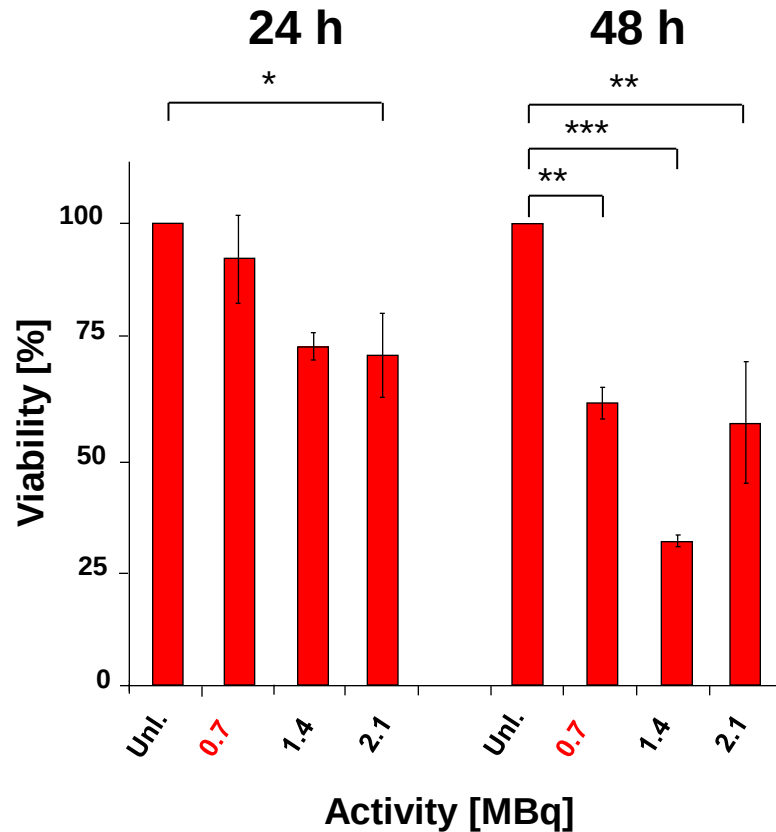


TCR-labelling: Ab against the TCR

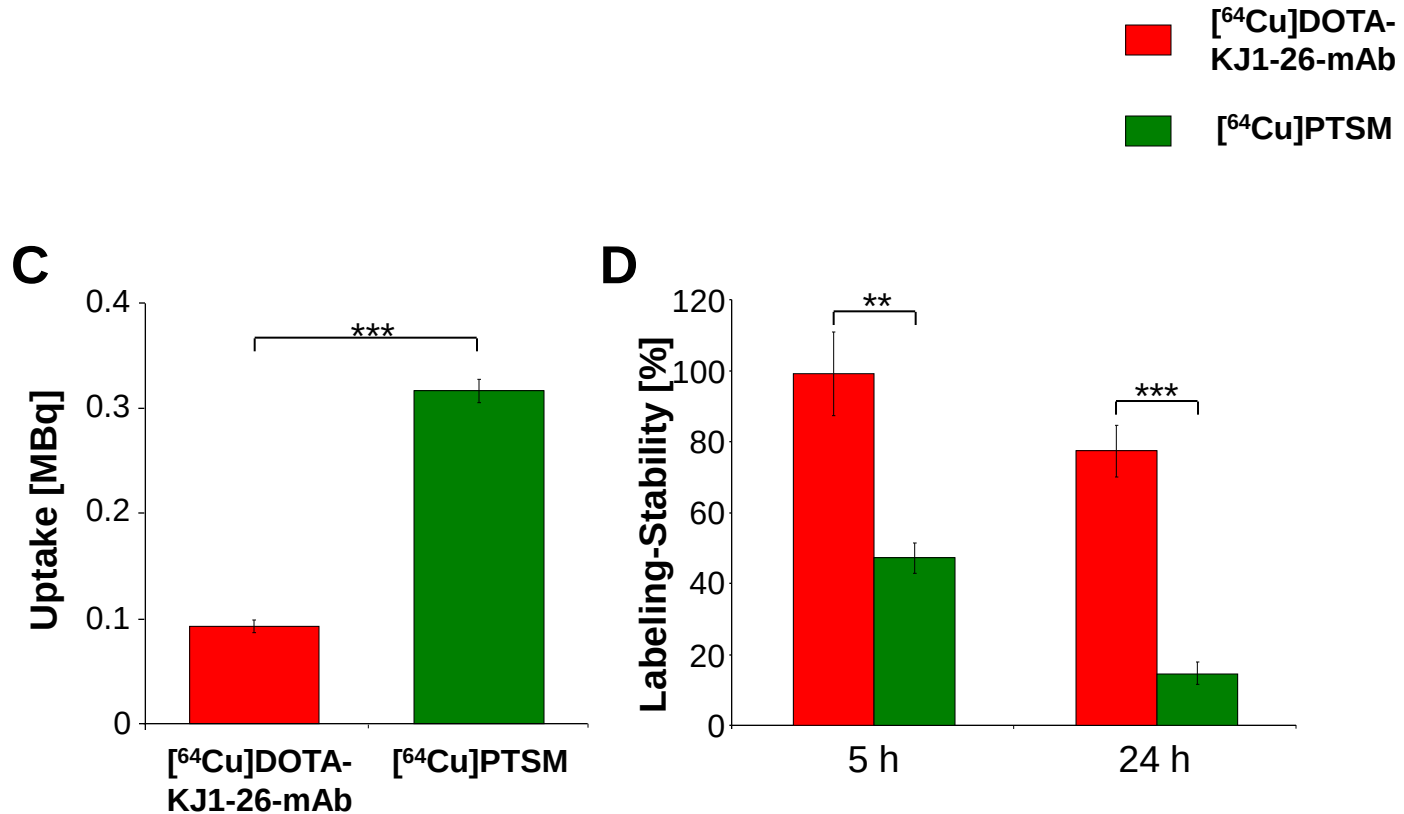
F(ab)-Fragment-labeled cOVA-TCRtg-Th1 cells



TCR-labelling: Ab against the TCR



TCR-labelling: Ab against the TCR



TCR-labelling: Ab against the TCR

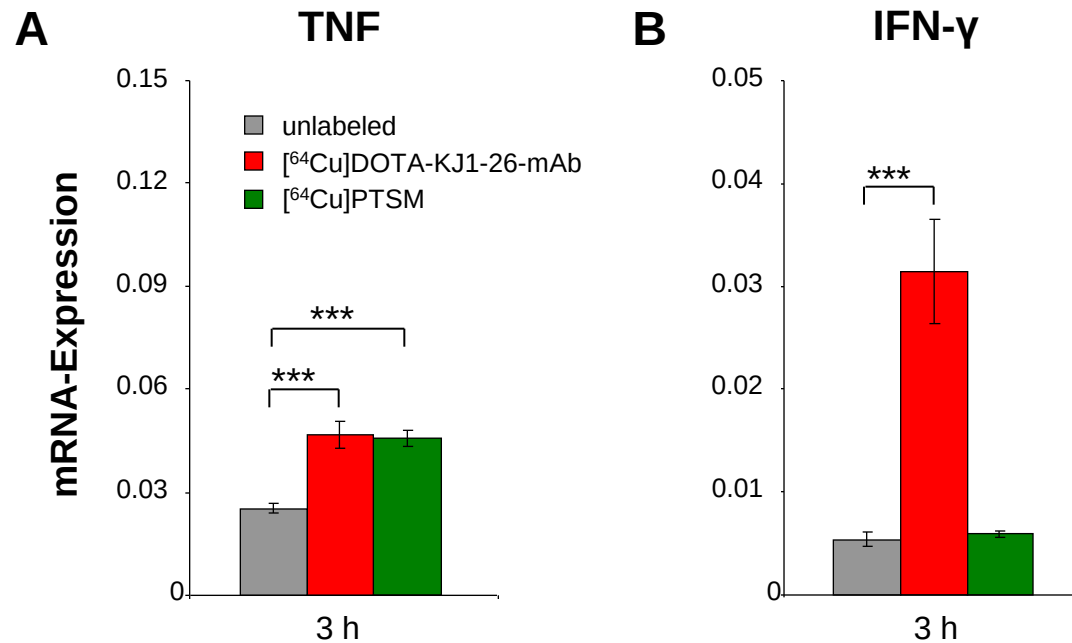




TABLE I

Cloned BALB/c-derived, cOVA-specific, I^d-restricted T Cell Hybridomas Used in These Studies

Hybridoma	IL-Production (U/ml) in response to A20-2J cells presenting these antigens							I-sub region restriction	Alloreactivity	
	cOVA	jfOVA	phOVA	tOVA	qOVA	wOVA	No antigen		IL-2	Specificity
									U/ml	
3DO-26.1	640	160	320	320	—	—	—	I-E ^d	—	none
3DO-36.6.1	80	320	—	640	80	—	—	I-E ^d	—	none
4DO-63.10	320	—	640	320	—	—	—	I-E ^d	—	none
3DO-54.8	640	320	—	—	160	—	—	I-A ^d	80	I-A ^{f,a}
									10	I-A ^k
3DO-54.6	640	160	—	—	—	—	—	I-A ^d	—	none
DO-11.10	640	320	—	—	—	—	—	I-A ^{d or b}	20	I-A ^b
3DO-18.3	640	640	320	—	320	—	—	I-A ^d	—	none
3DO-46.4	320	160	320	—	—	—	—	I-A ^d	—	none
3DO-20.10	640	—	—	—	—	—	—	I-A ^d	—	none
4DO-11.7	640	—	—	640	—	320	—	I-A ^d	—	none

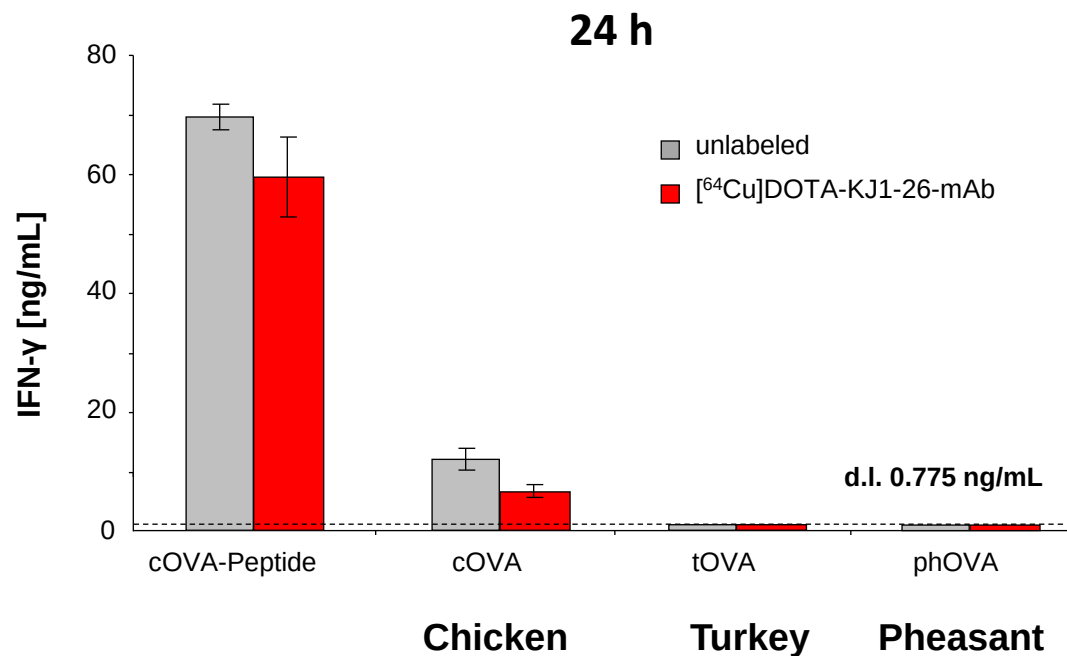
Antigen stimulation cultures and IL-2 assay performed as described in the Materials and Methods. Data presented is averaged from numerous assays over a several week period. Variance of IL-2 responses are $\pm$ one dilution except for negative responses which were never positive.

Shimonkevitz R et al, J Exp Med. 1983

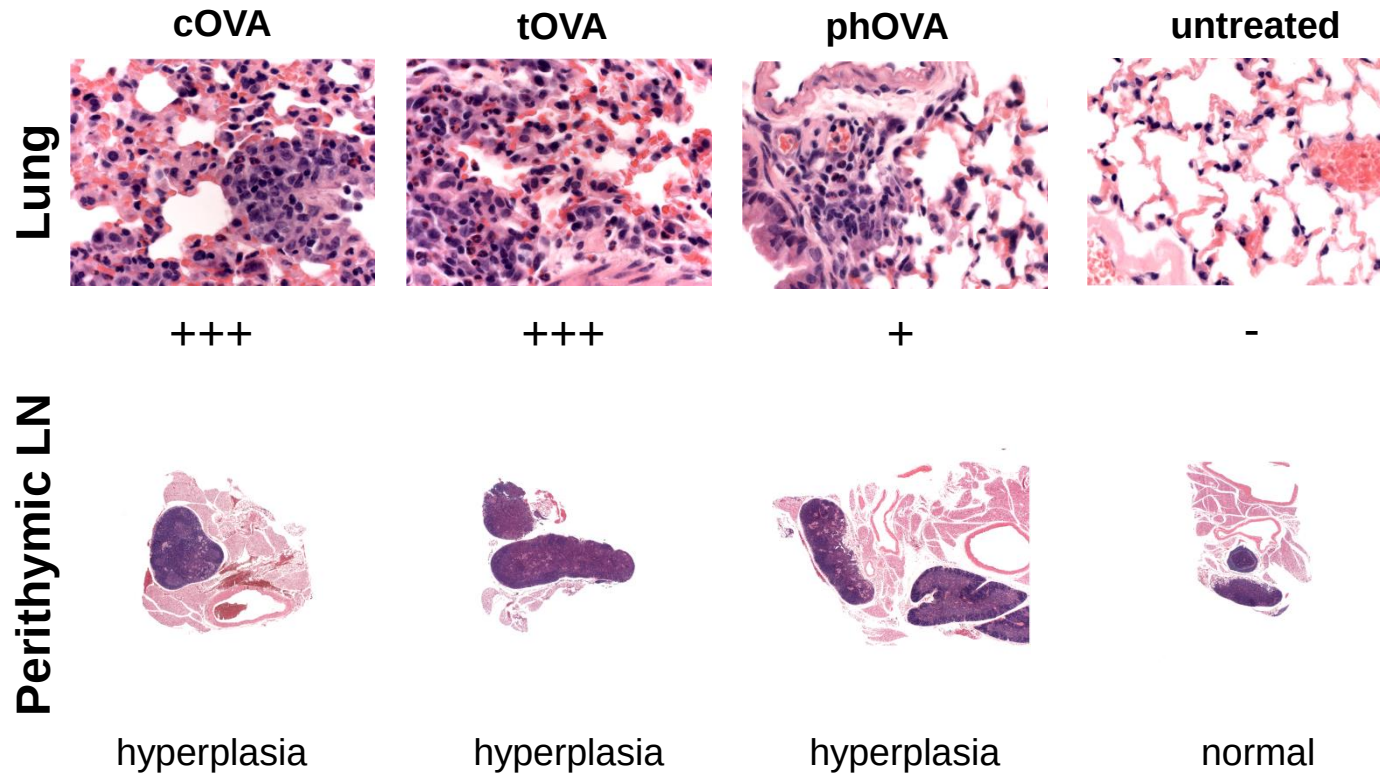


Shimonkevitz R et al, J Exp Med. 1983

Reproducible?

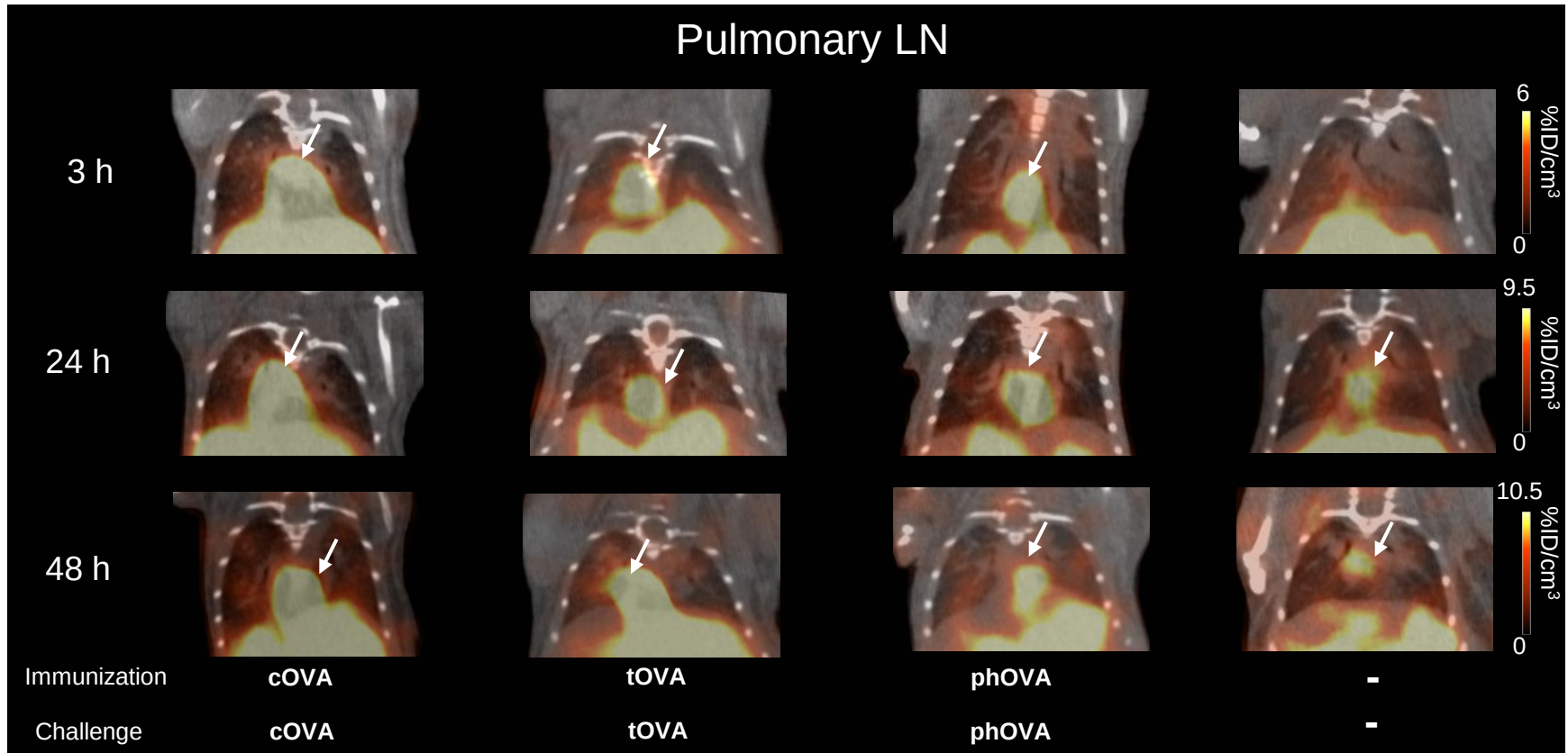


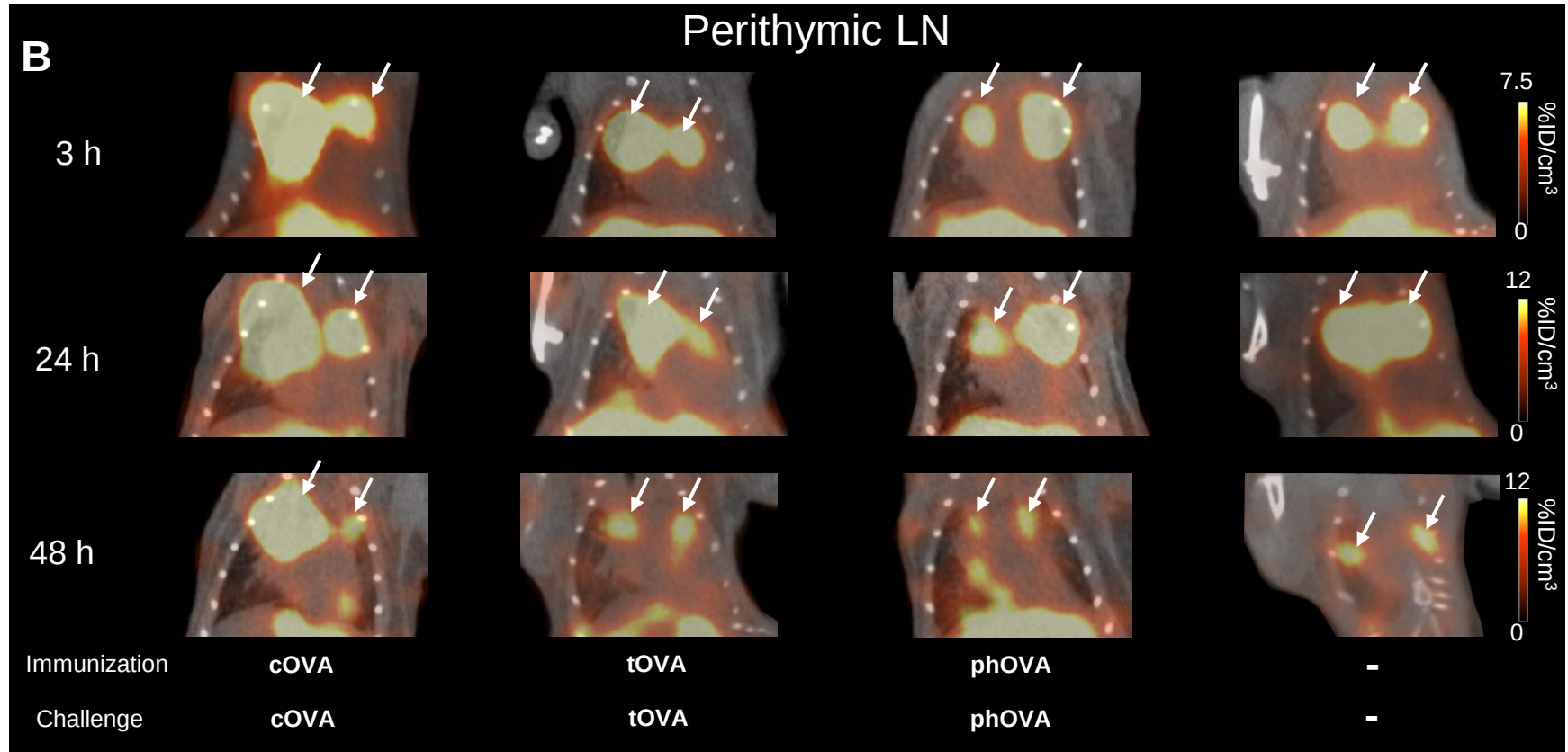
OVA-Th1 cells Specific Homing?



OVA-Th1 cells Specific Homing?

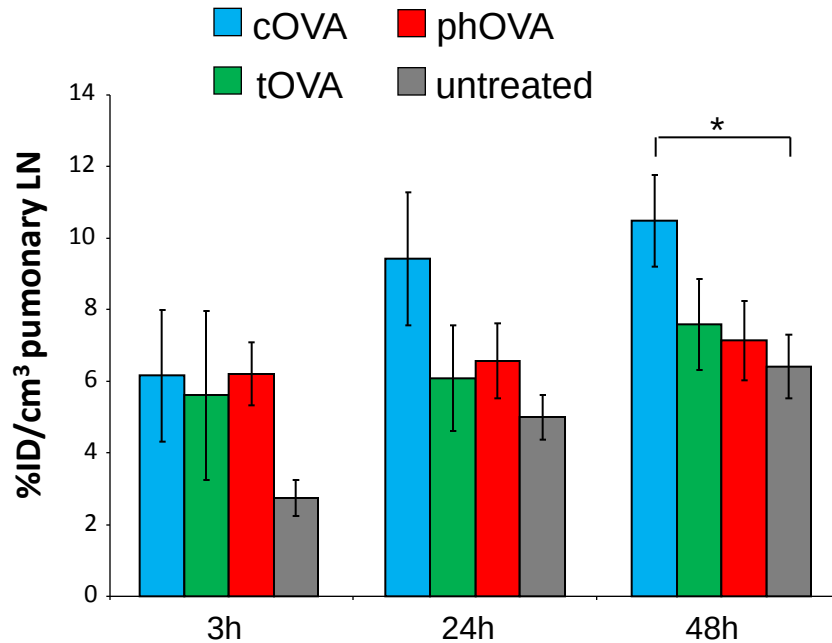
Pulmonary LN



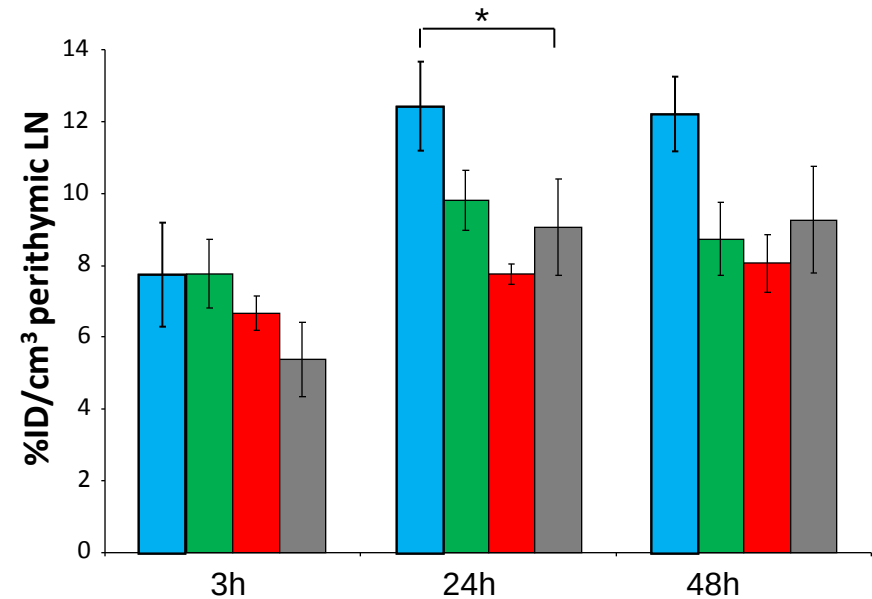


OVA-Th1 cells Specific Homing?

Yes!



Yes!



⁶⁴Cu antibody-targeting of the T-cell receptor and subsequent internalization enables in vivo tracking of lymphocytes by PET

Christoph M. Griessinger^a, Andreas Maurer^a, Christian Kesenheimer^a, Rainer Kehlbach^b, Gerald Reischl^a, Walter Ehrlichmann^a, Daniel Bukala^a, Maren Harant^a, Funda Cay^a, Jürgen Brück^c, Renate Nordin^c, Ursula Kohlhofer^d, Hans-Georg Rammensee^{e,f}, Leticia Quintanilla-Martinez^d, Martin Schaller^c, Martin Röcken^c, Bernd J. Pichler^{a,1}, and Manfred Kneilling^{a,c}

^aDepartment of Preclinical Imaging and Radiopharmacy, Werner Siemens Imaging Center, ^bDepartment of Diagnostic and Interventional Radiology, ^cDepartment of Dermatology, ^dDepartment for Pathology, and ^eDepartment of Immunology, Eberhard Karls University, 72076 Tübingen, Germany; and ^fGerman Cancer Consortium, German Cancer Research Center Partner Site Tübingen, 72076 Tübingen, Germany

Edited by Owen N. Witte, Howard Hughes Medical Institute, University of California, Los Angeles, CA, and approved December 22, 2014 (received for review September 24, 2014)

January 2015

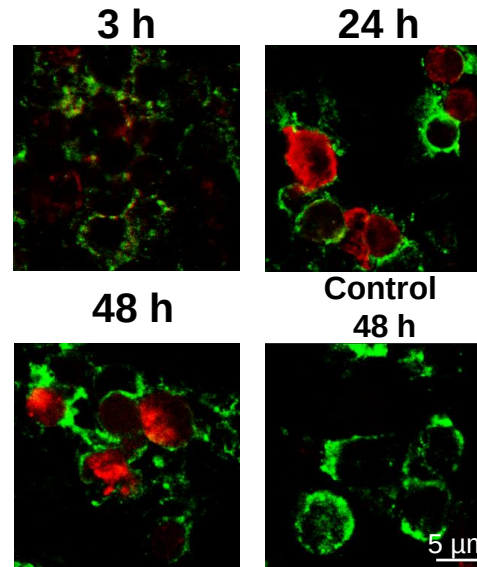
Is this method applicable to other TCRs / cell types?

Other TCRtg Tcells and cell types

Tag2-TCRtg T cells

RIP1-Tag2-pancreatic cancer model

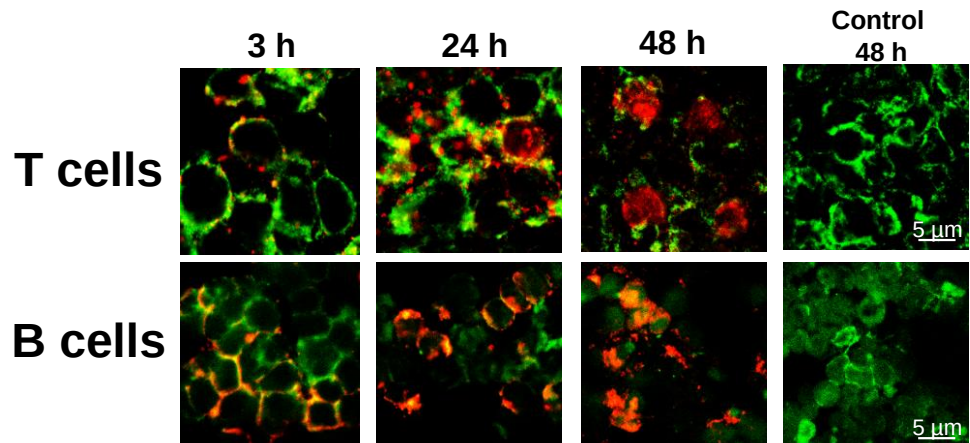
(9H5.1 Ab specific for large T antigen (Tag2) T cell receptor)



B- and T cells

Targeting Thy1.2

(53-2.1 Ab specific for Thy1.2 (CD90.2) expressed on lymphocytes)



Griessinger et al., PNAS 2015

mAb-labeling allows to follow the homing dynamics of cells of interest

- limited impairments of the labeled cells
- Low background

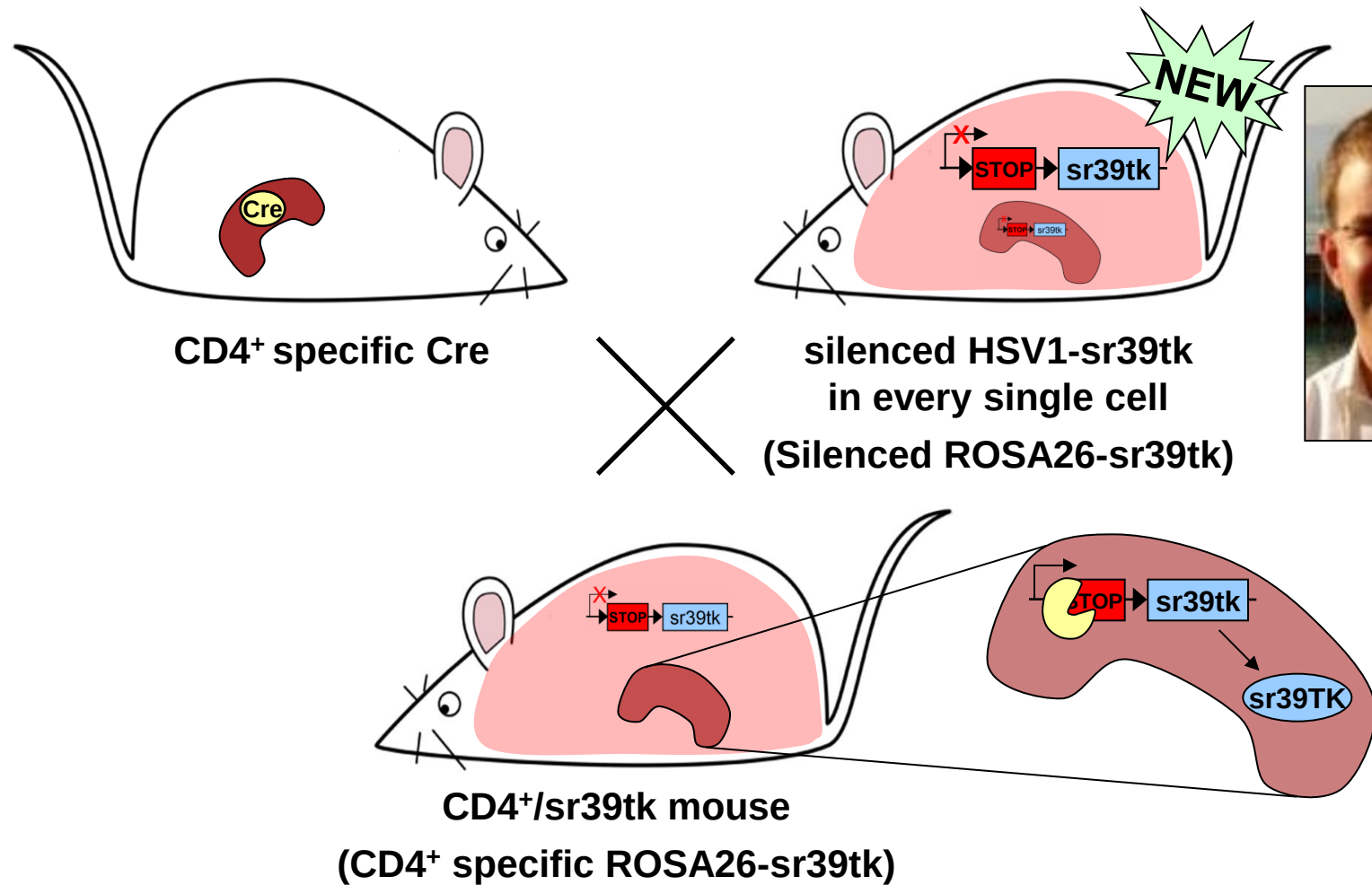
Our mAb labeling strategy can be easily transferred to other T cells as well as other immune cell types:

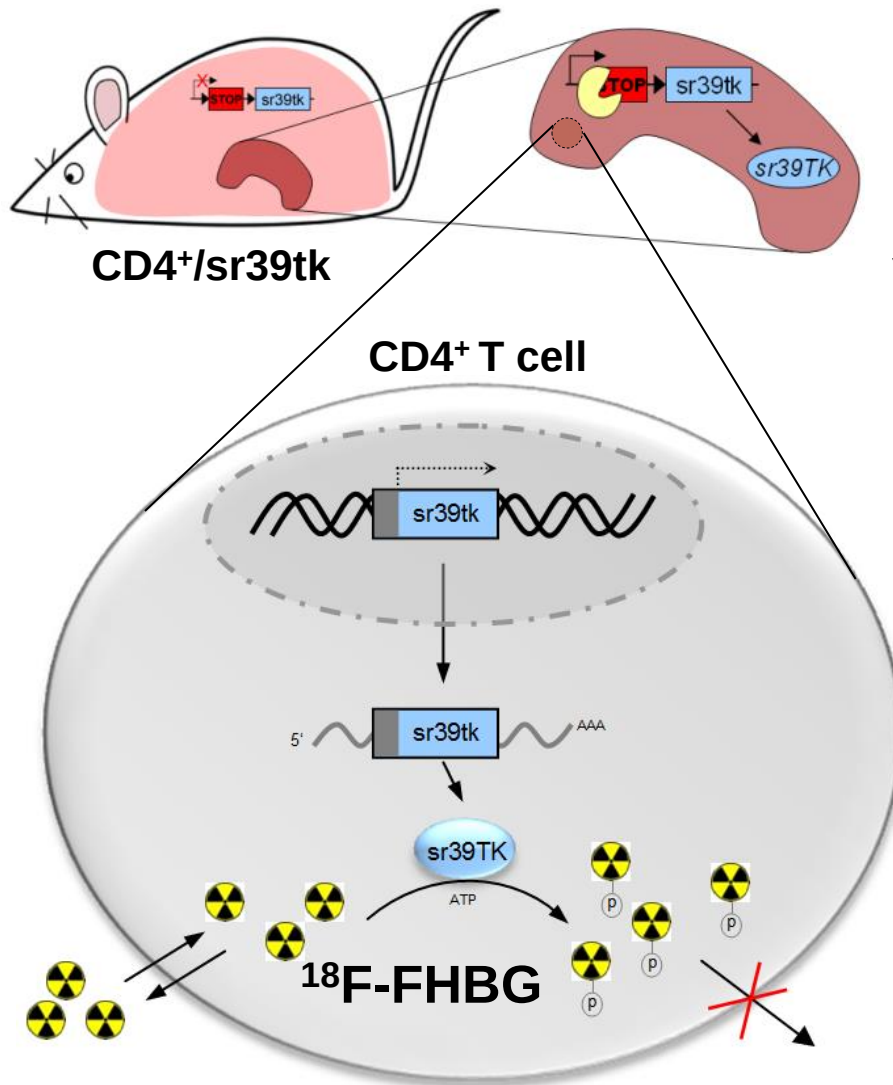
- **T-cells** (OVA^{tg}-TCR, Tag2^{tg}-TCR, Thy1.2)
- **B cells** (Thy1.2)
- **Macrophages/Granulocytes** (CD11b)

Ab-induced effects on the cell type of interest has to be analyzed prior to the imaging studies

- **[⁶⁴Cu]DOTA-Ab-labeling** enables visualization of specific Th1 cell homing patterns.

Introduction: Generation of CD4⁺/sr39TK Mice





Phosphorylation of radiolabeled nucleoside analog PET reporter probe (¹⁸F-FHBG/¹²⁴I-FIAU) by sr39tk (reporter gene)

→ ¹⁸F-FHBG trapping in the T cell

Tracer affinity:

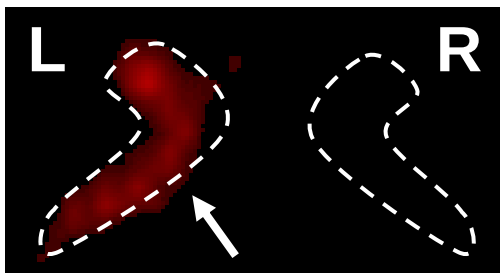
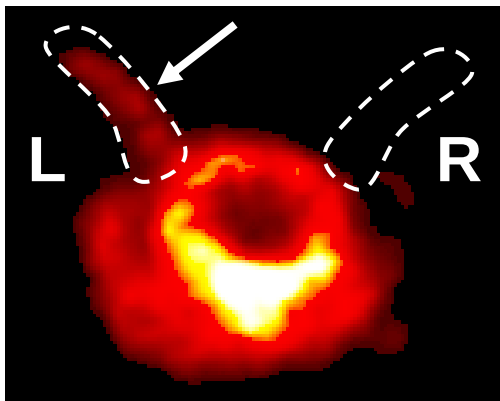
HSV1-sr39tk > HSV1-tk



Results I: CD4⁺/sr39tk Mice with CHS

PET/MR of endogenous sr39tk-expressing CD4⁺ mouse with CHS at the ear

PET (Ears)



¹⁸F-FHBG-Uptake:

CD4⁺

- Left inflamed ear with chronic CHS

→ Specific homing of endogenous CD4⁺ T cells



- **CD4⁺/sr39TK Mice** enables longitudinal visualization of endogeneous CD4⁺ T cell homing patterns.



ARTICLE

DOI: 10.1038/s41467-017-00482-y

OPEN

Cre/lox-assisted non-invasive in vivo tracking of specific cell populations by positron emission tomography

Martin Thunemann^{1,8}, Barbara F. Schörg², Susanne Feil¹, Yun Lin², Jakob Voelkl³, Matthias Golla¹, Angelos Vachaviolos¹, Ursula Kohlhofer⁴, Leticia Quintanilla-Martinez⁴, Marcus Olbrich⁵, Walter Ehrlichmann², Gerald Reischl², Christoph M. Griessinger², Harald F. Langer⁵, Meinrad Gawaz⁵, Florian Lang³, Michael Schäfers⁶, Manfred Kneilling^{2,7}, Bernd J. Pichler² & Robert Feil¹



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Department of Dermatology, Tübingen: Prof. Martin Röcken,
Prof. Tilo Biedermann, Prof. Martin Schaller, Dr. Heidi Braumüller,
Dr. Susanne Kaessler, Birgit Fehrenbacher

Department for Pathology, Tübingen: Dr. Leticia Quintanilla de Fend, Dr. Ursula Kohlhofer

IFIB, Tübingen: Dr. Hubert Kalbacher



GEFÖRDERT VOM



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