陕西师范大学
SHAANXI NORMAL UNIVERSITY

研究生教育教学改革研究项目
（研究生优质课程项目）

生物实验室安全及大型仪器应用

小动物活体成像系统

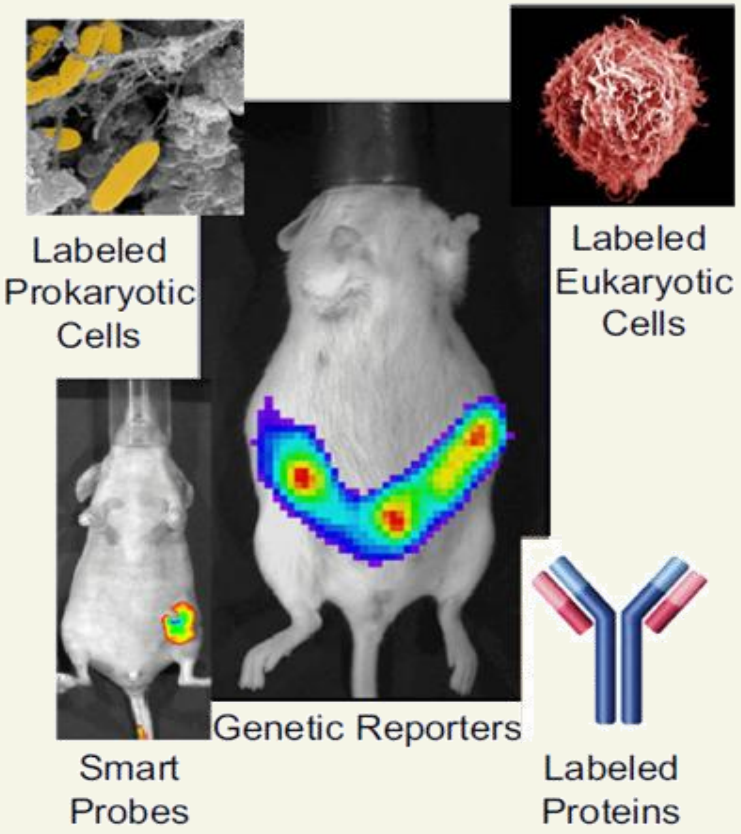
主讲教师 郑晓晶

生命科学学院实验教学中心

活体光学成像——技术核心

小动物活体光学成像——是通过一定的方式对研究对象进行光学标记，使其具有发光的性质，再通过成像技术及设备对光信号进行采集成像。

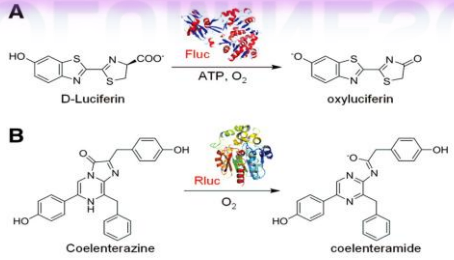
光学标记

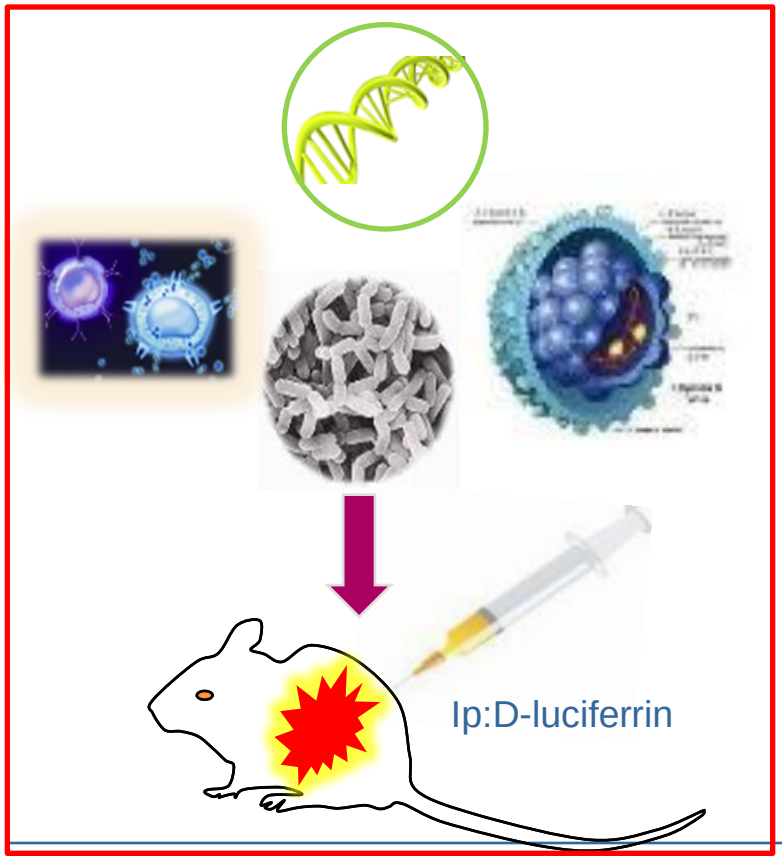


成像技术及设备

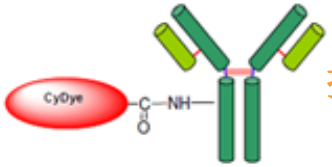
技术原理——标记方式

BIOLUMINESCENCE

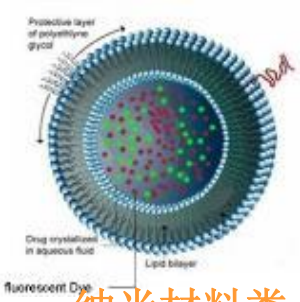




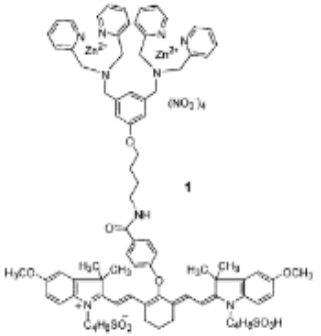
FLUORESCENCE



抗体、蛋白类

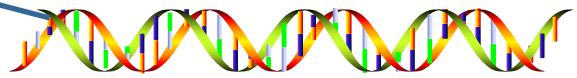


纳米材料类

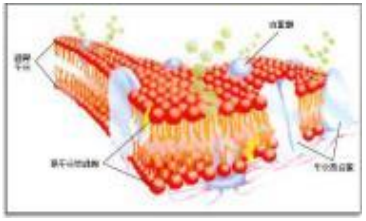


化合物

染料

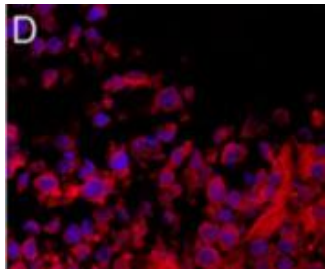
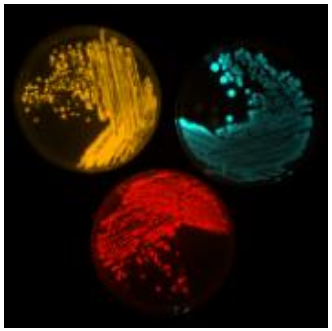
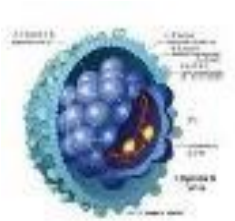
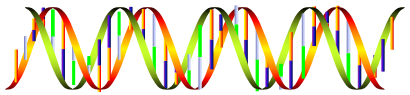


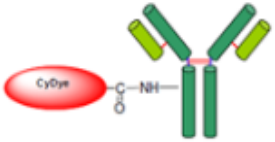
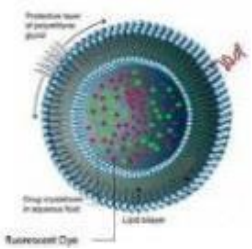
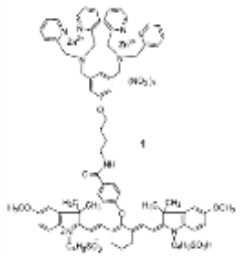
siRNA



细胞

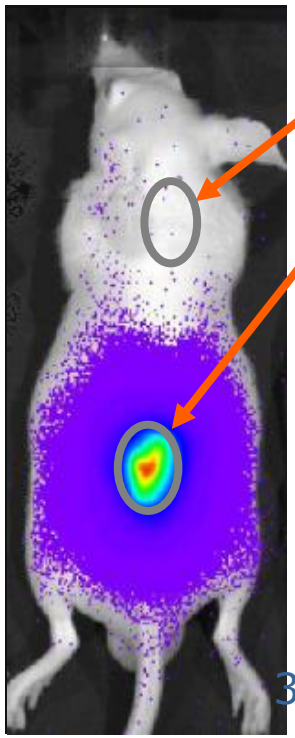
标记方式-小结

检测对象	标记方式
	荧光素酶（长期） 荧光蛋白（长期） 脂溶性荧光染料（短期） 荧光探针
	荧光素酶（长期） 荧光蛋白（长期） 荧光探针
	荧光素酶 荧光蛋白
	荧光素酶 荧光蛋白

检测对象	标记方式
	活化荧光染料-共价结合
	荧光染料-包裹、包埋、共价结合
	活化荧光染料-共价结合
	活化荧光染料-共价结合

技术原理——生物发光 vs 荧光

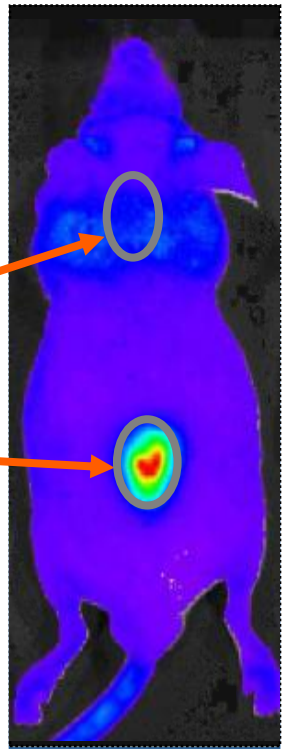
生物发光成像



背景信号强度 ~ 9.5×10^3 p/s
光信号强度 ~ 7.1×10^7 p/s
信噪比 ~ 7500

背景信号强度 ~ 1.0×10^9 p/s
光信号强度 ~ 7.8×10^9 p/s
信噪比 ~ 7.8

荧光成像

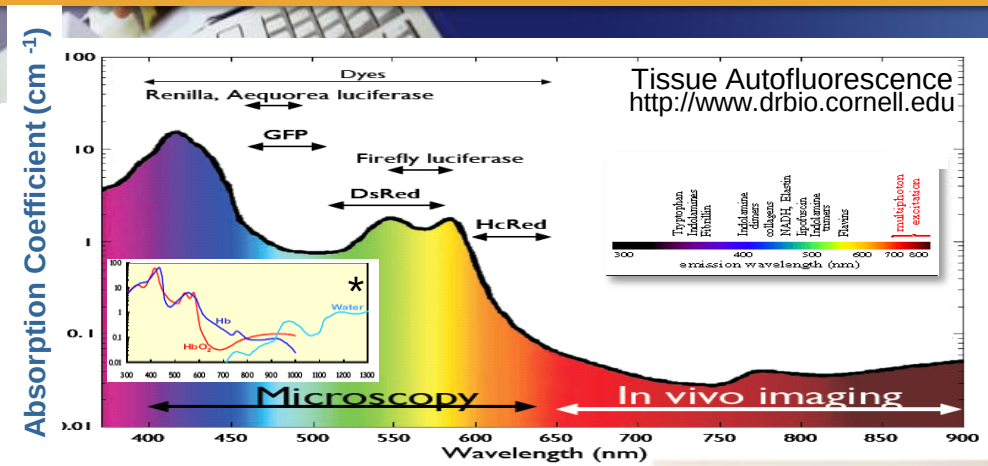


3×10^6 PC3M-luc/DsRed cells injected s.c.

荧光成像灵敏度通常比生物发光成像低1000倍

T.L. Troy, et al. (2004). Molecular Imaging 3:9-23

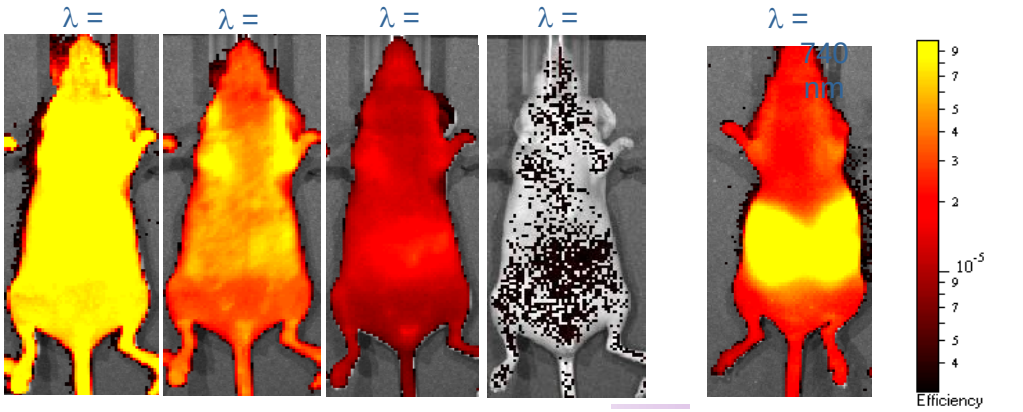
波长选择—透过率和背景噪音



*http://ase.tufts.edu/biomedical/research/Fantini

Depilation

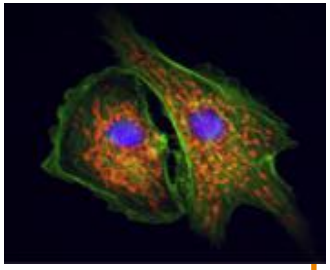
SNR=Signal/Autofluorescence

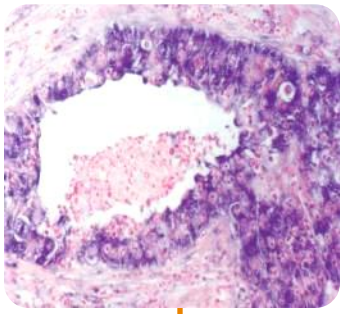


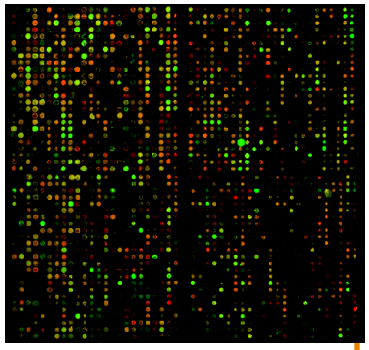
Alfalfa Free Rodent Food

Regular Food

应用优势——体内研究的重要工具







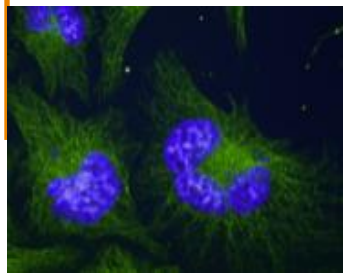
Organism

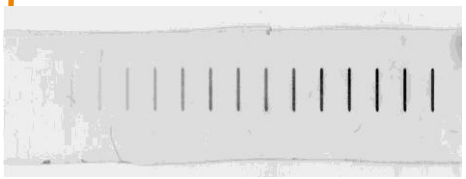
Cell

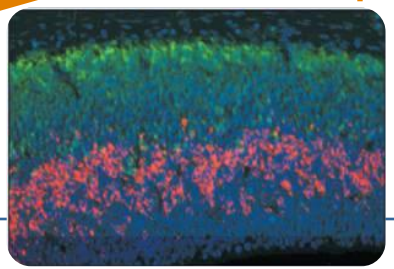
Protein

RNA

DNA



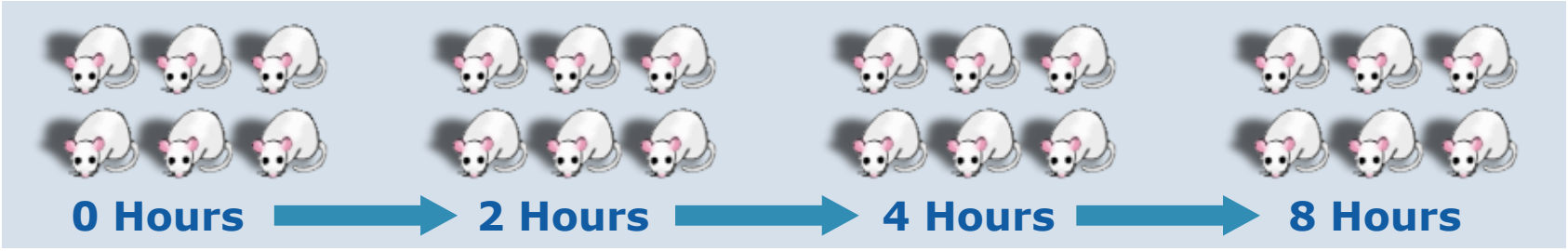




应用优势——降低成本、缩减时间、提高数据精确度及重复性

Current Methodology = 24 animals over four treatment points

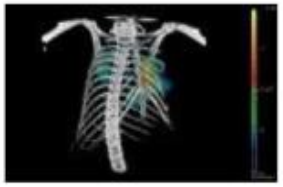
BPI Methodology = the same 6 animals over four treatment points



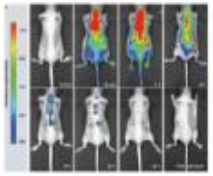
Same group of anesthetized test animals at each time point of an experiment uses far fewer animals than current methodology

应用方向概览

肿瘤癌症

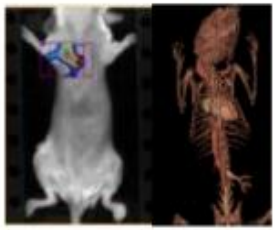


纳米材料研究



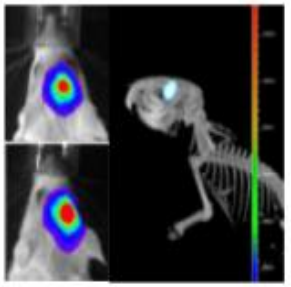
炎症

心血管疾病

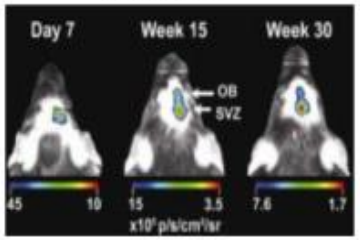


药物研究

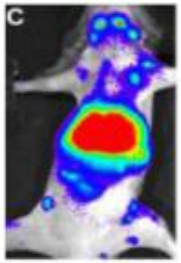
神经科学



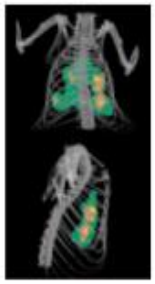
干细胞研究



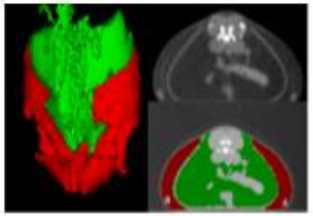
免疫和移植生物学



传染性疾病



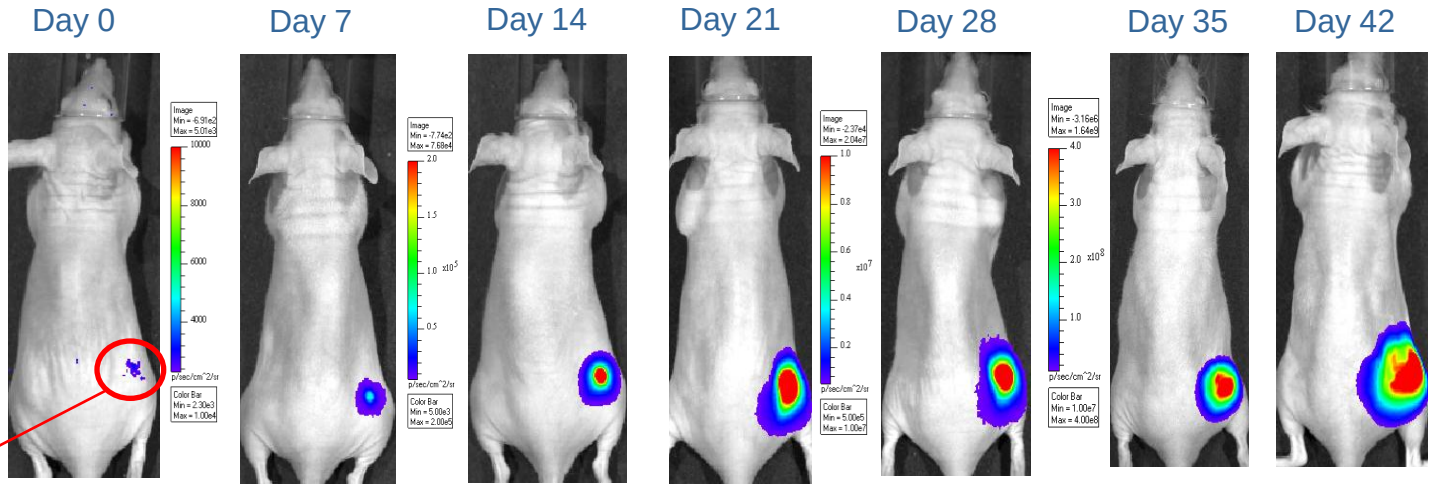
代谢研究

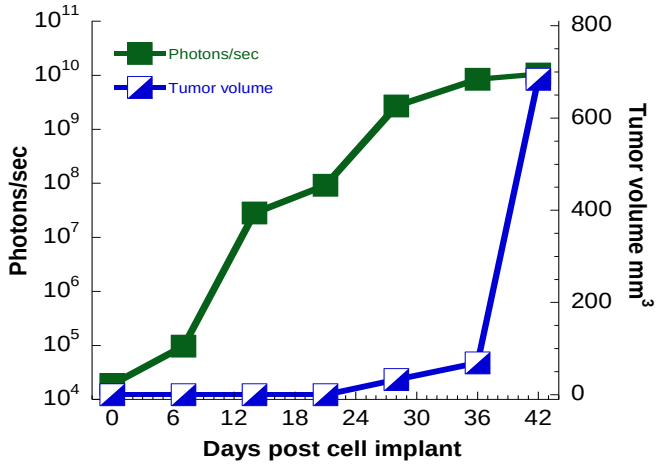


肿瘤相关研究

长期检测肿瘤的生长—“看的早”

Bioware Ultra: 4T1-luc2





With Bioware Ultra one can start collecting data from Day 0, while with caliper measurements one has to wait at least 28 days to see any tumor growth

荧光探针检测肿瘤

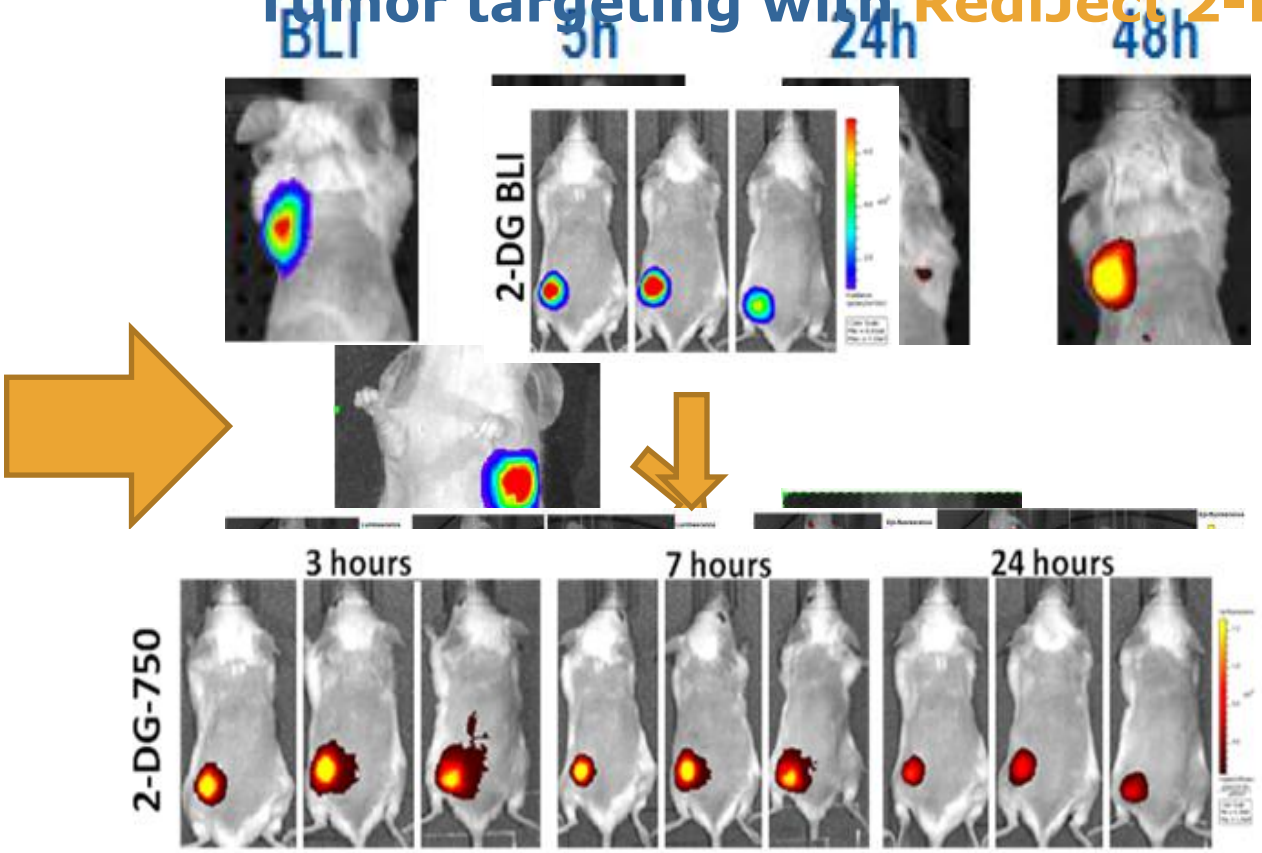
Functional Fluorescent Agents

- Her2Sense
- PSA FAST
- HypoxiSense
- IntegriSense
- BombesinRSense
- Annexin Vivo
- FolateRSense
- ProSense
- ProSense FAST
- CatB FAST
- MMPSense
- MMPSense FAST
- AngioSPARK
- AngioSense
- 2DG-750

AngioSense 680 EX

SKOV-3 tumor-bearing mice injected i.v. with 40 mg Her2Sense 645

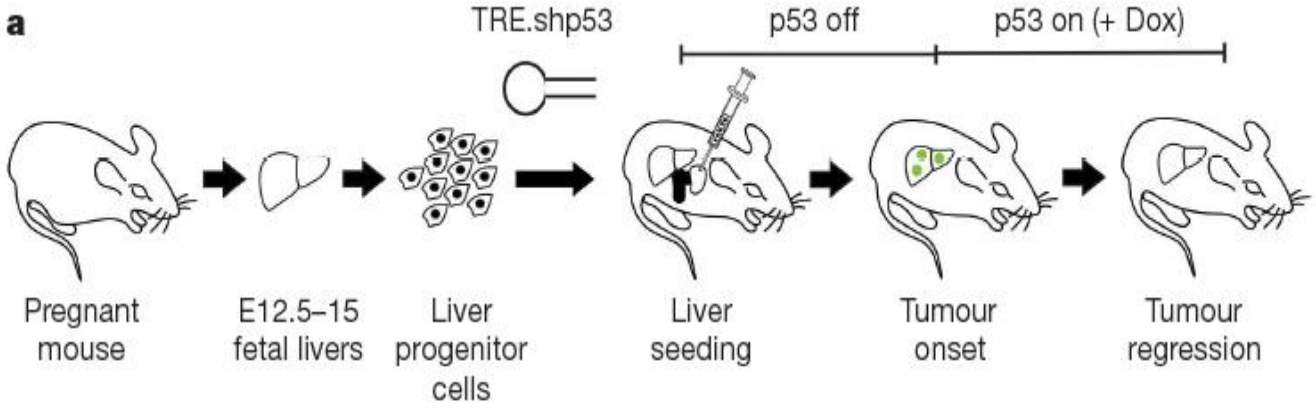
Tumor targeting with RediJect 2-D



H129 tumor-bearing mice were injected i.v. with 2 nmoles BombesinRSense 680 and imaged 24 h later.

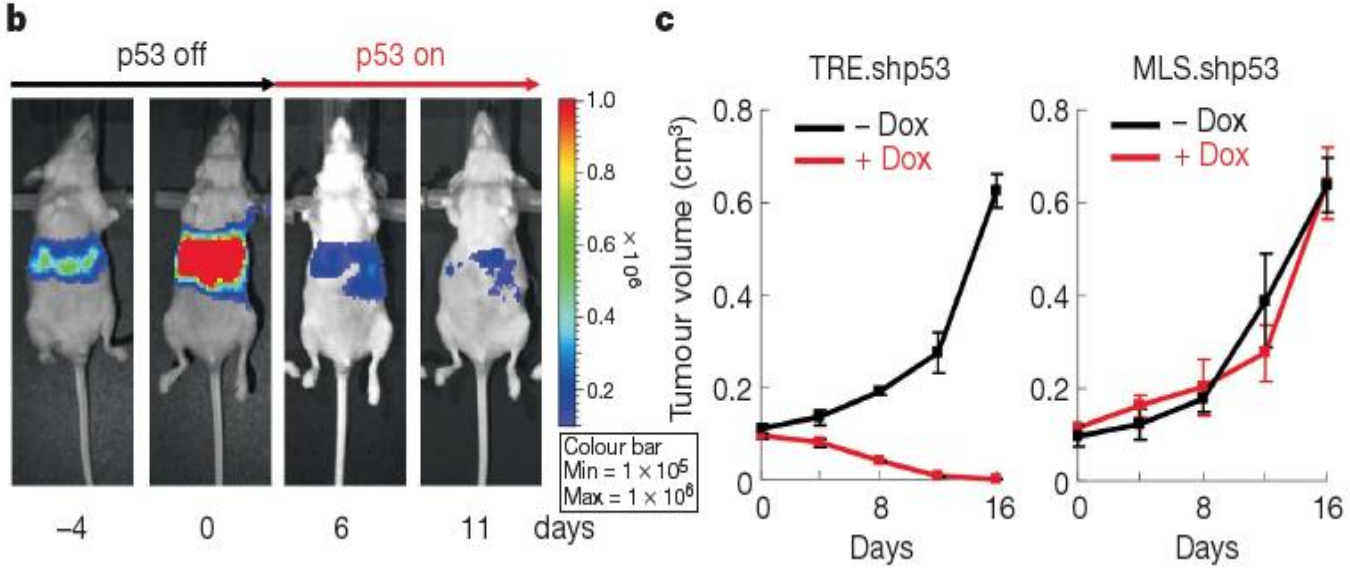
ProSense 750 EX

p53重新激活导致肝脏肿瘤的退化



强力霉素
- doxycycline

ras ↑ ↓ p53
(p53 shRNA)



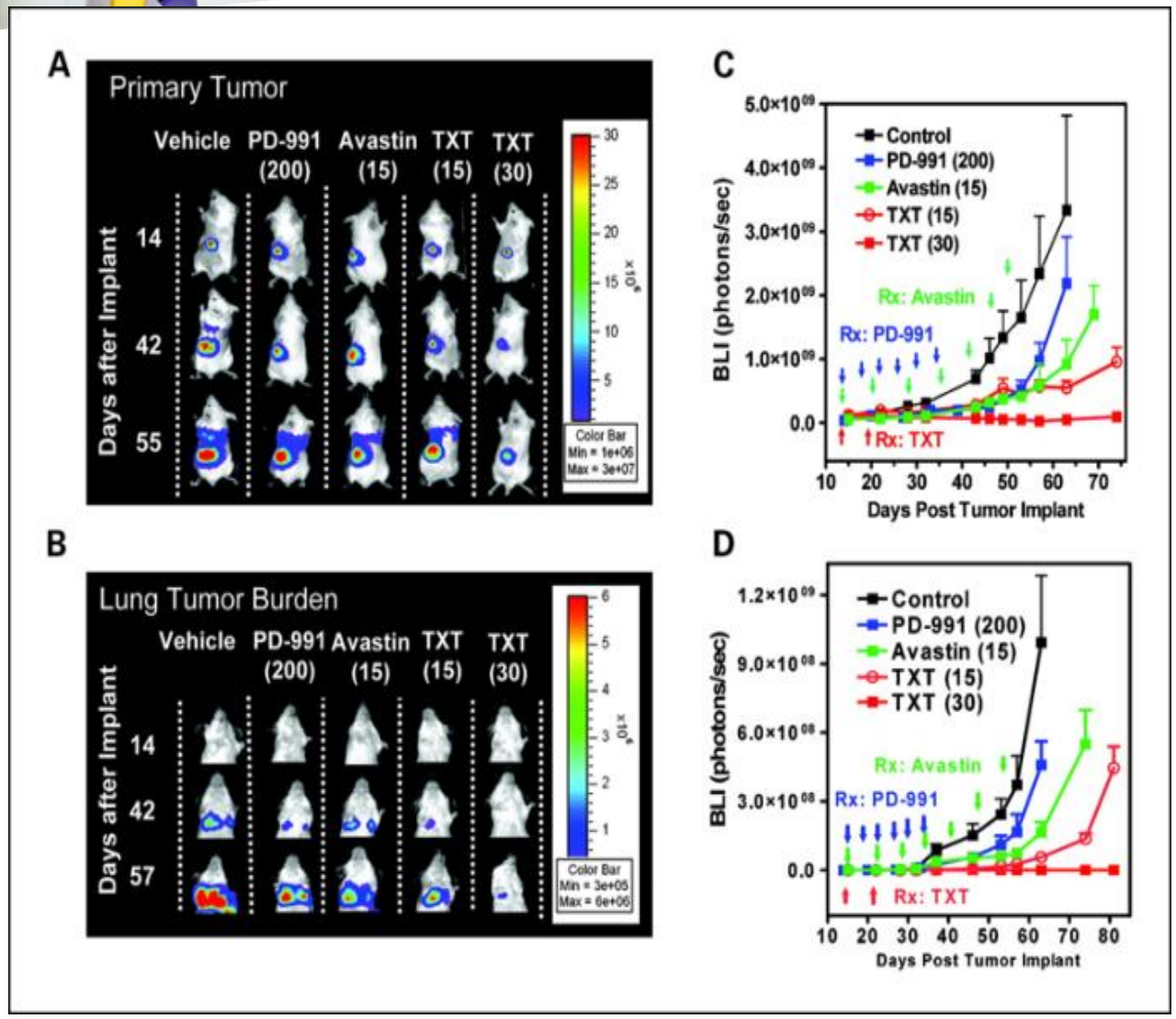
+ doxycycline

ras ↑ ↑ p53
(tet-off,
p53 shRNA)

Triggering upregulation
of inflammatory cytokines
& the innate immune system

Xue et al, Nature, 2007

生物发光成像药物治疗效果

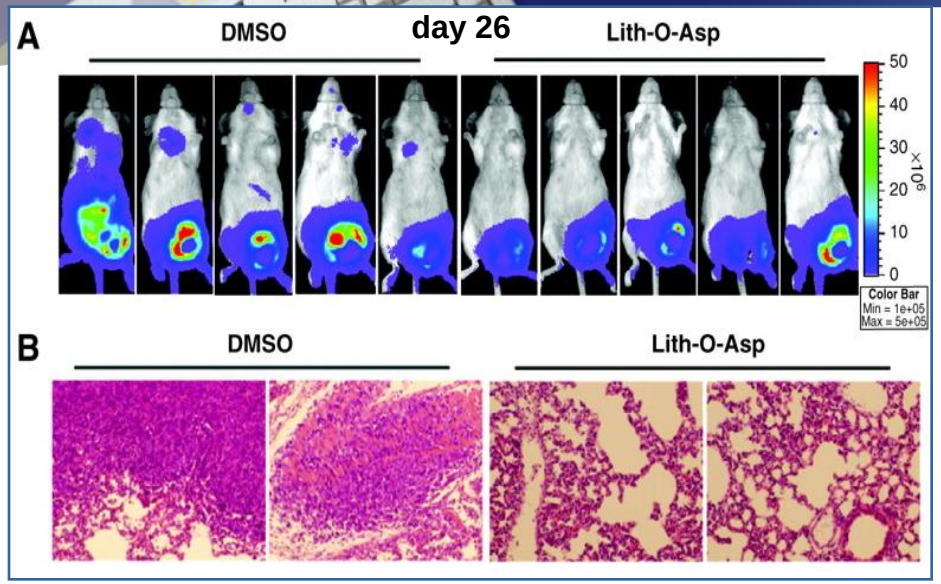


Benefits

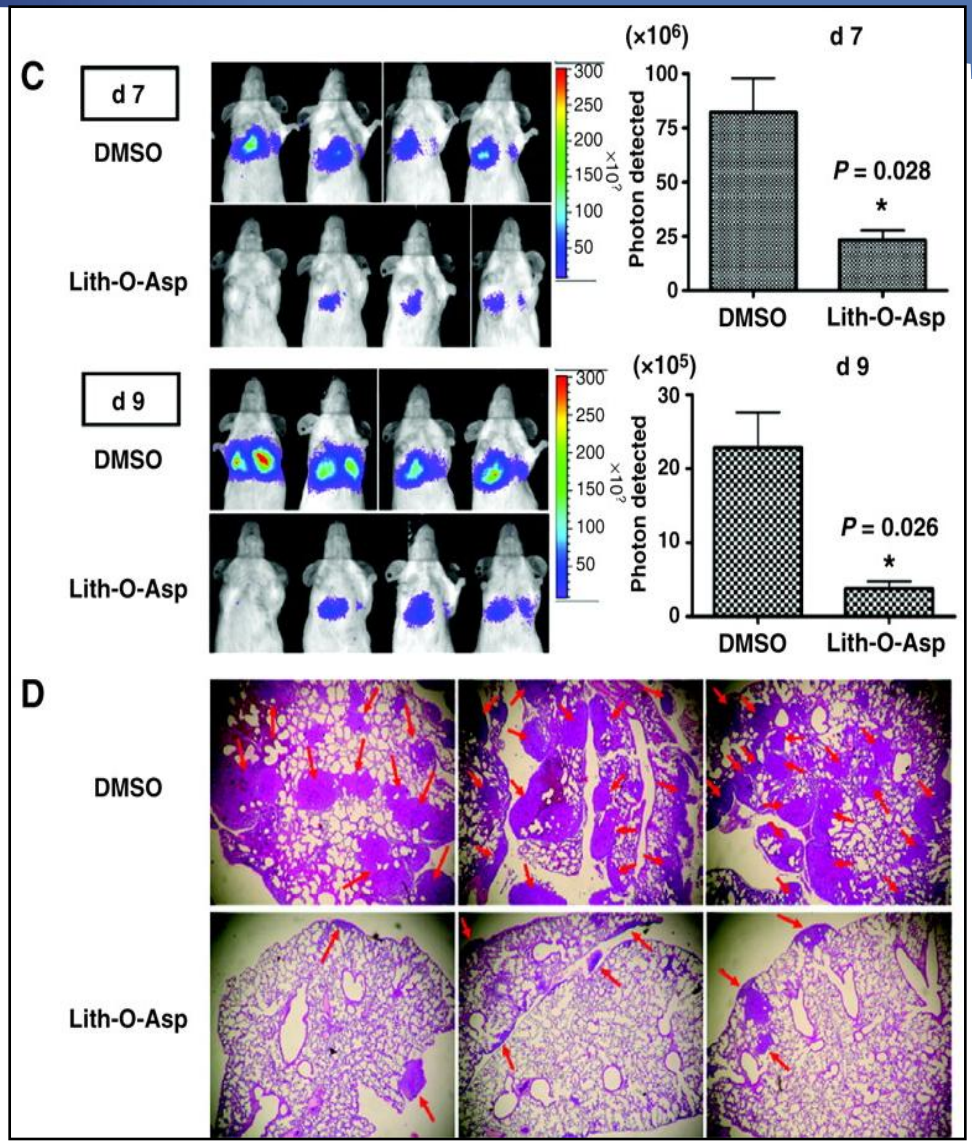
- Expedites progress towards clinical trials
- Measurable economic benefits include reduced animal costs and personnel
- Simultaneous insights into drug efficacy, kinetics, target, mechanism
- Superior statistics and data reproducibility

Zhang et al, Clin Cancer Res., 2009

乳腺癌转移治疗策略(唾液酸转移酶抑制剂)



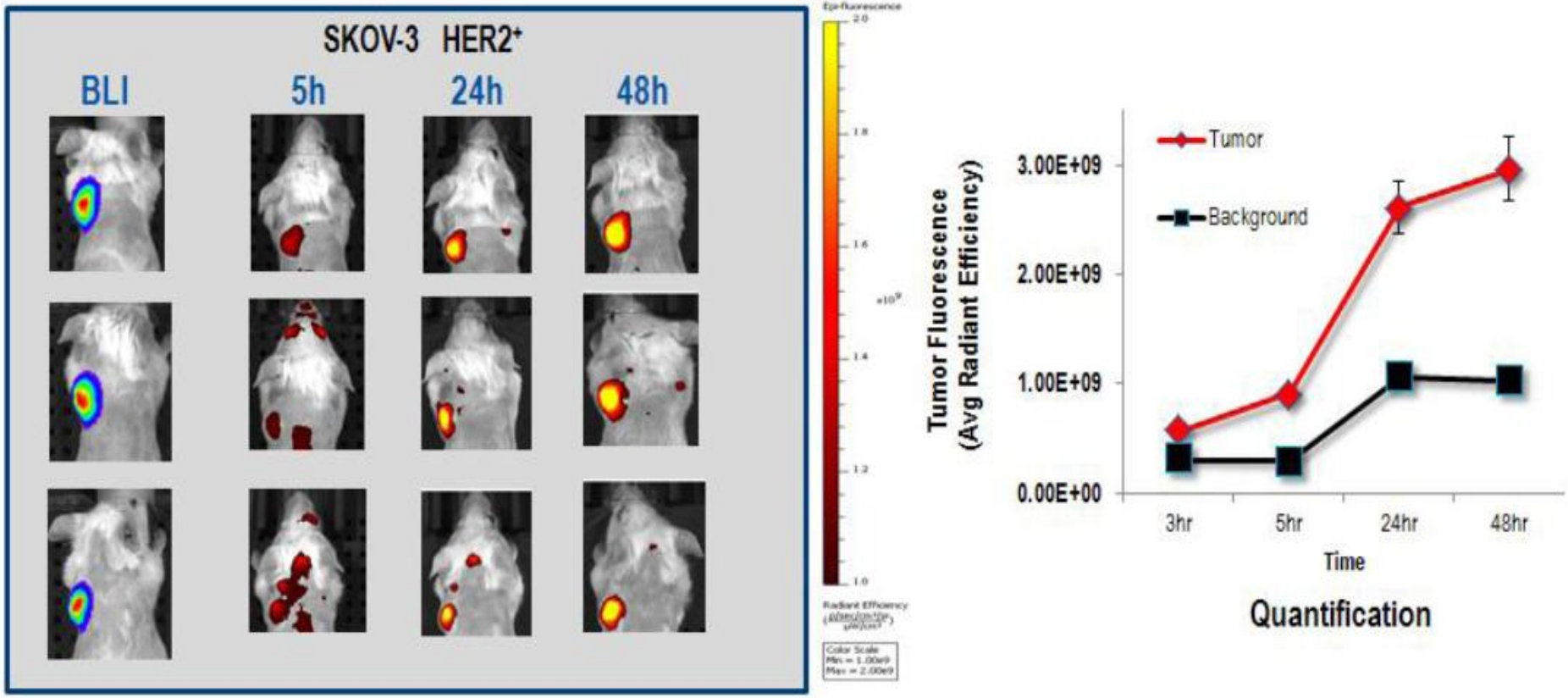
- 唾液酸转移酶(ST) 激活促进肿瘤转移；过表达细胞表面的唾液酸。而唾液酸与癌症病人预后效果差有直接关系。
- 设计合成新型的ST抑制剂，Lith-O-Asp, 抑制乳腺癌转移并且抑制phospho-FAK, phospho-paxillin, MMP2 and MMP9的表达。
- 5×10^5 4T1-luc细胞乳房脂肪垫接种。



Chen *et al*, Clin. Cancer Res., 2011

1×10^5 4T1-luc细胞lith-o-Asp预处理后尾静脉注射

药物的肿瘤靶向性研究

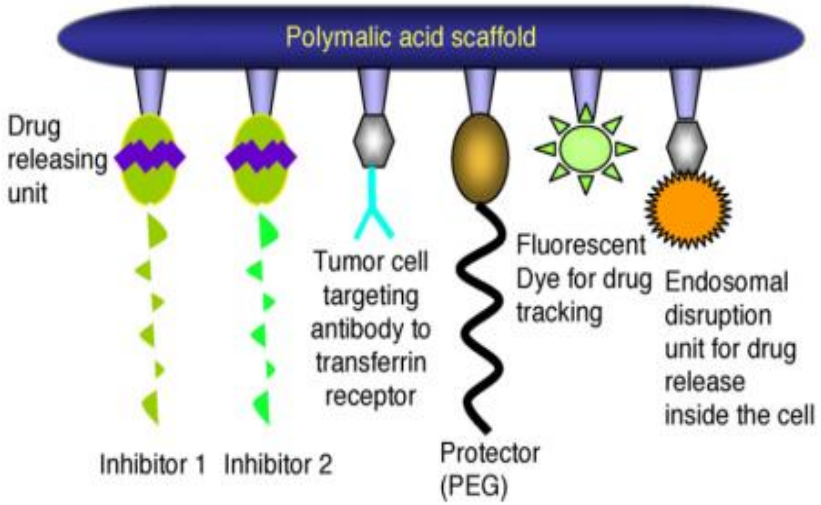


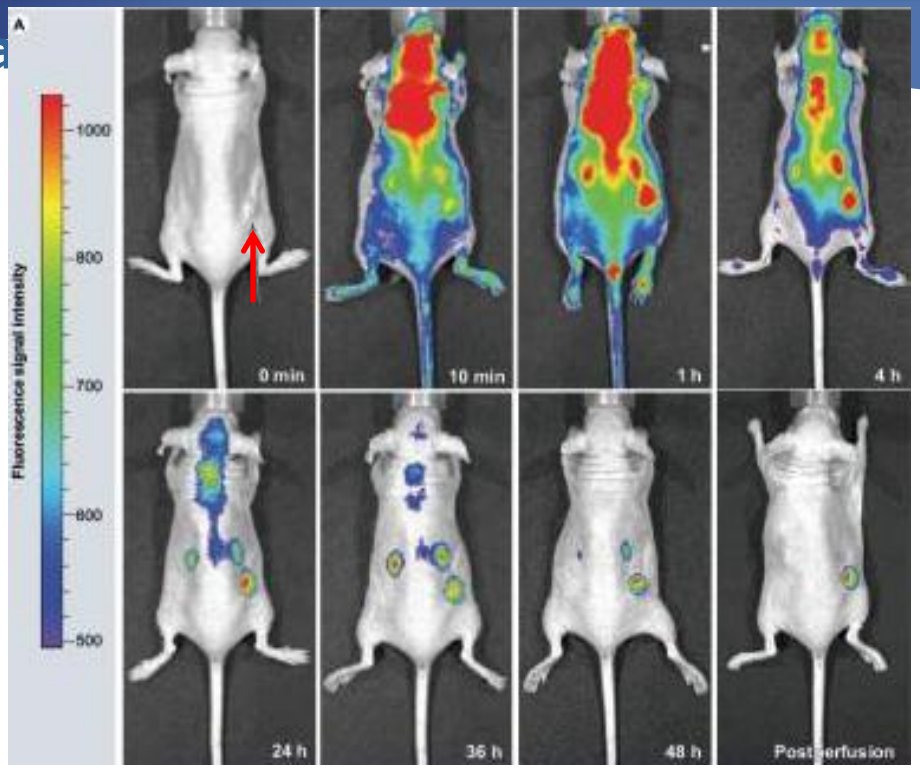
VivoTag 645荧光染料标记抗癌药物曲妥珠单抗(Trastuzumab)

HER2阳性人卵巢癌SKOV3的SCID小鼠

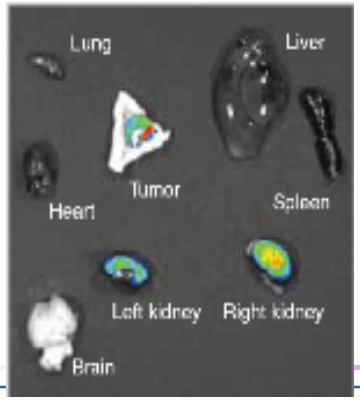
新型纳米共聚物载药系统

Poly (malic acid) nanoconjuga





- 新型的载药系统系统包含几个功能原件；
- 链接荧光- AlexaFluor 680
- 转铁蛋白受体的抗体- 肿瘤细胞过表达

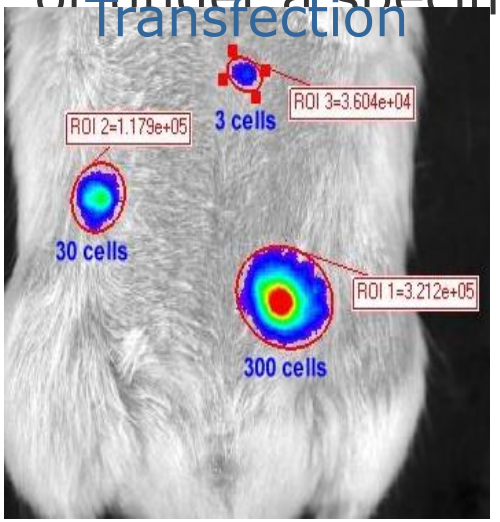


Ljubimova et al., Nanomedicine 2008

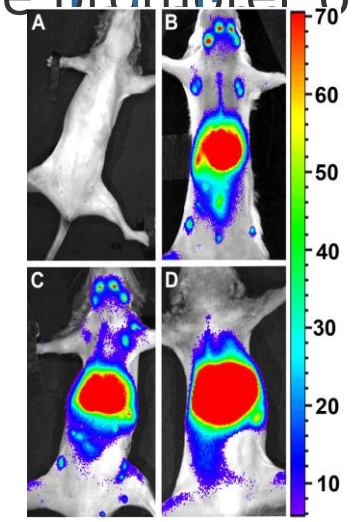
免疫细胞标记方法

- Stable transduction of murine and human immune cell populations with viral vectors carrying a luciferase/fluorescent reporter gene
- Short-term labeling with fluorescent dyes (e.g. DiR, DiD etc.)
- Isolation of immune cells from a transgenic mouse/rat expressing luciferase under the β-actin promoter (ubiquitous) or under a specific gene promoter of interest (inducible)

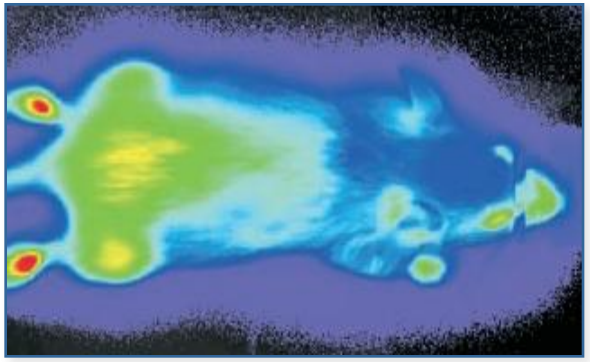
Stable Transfection



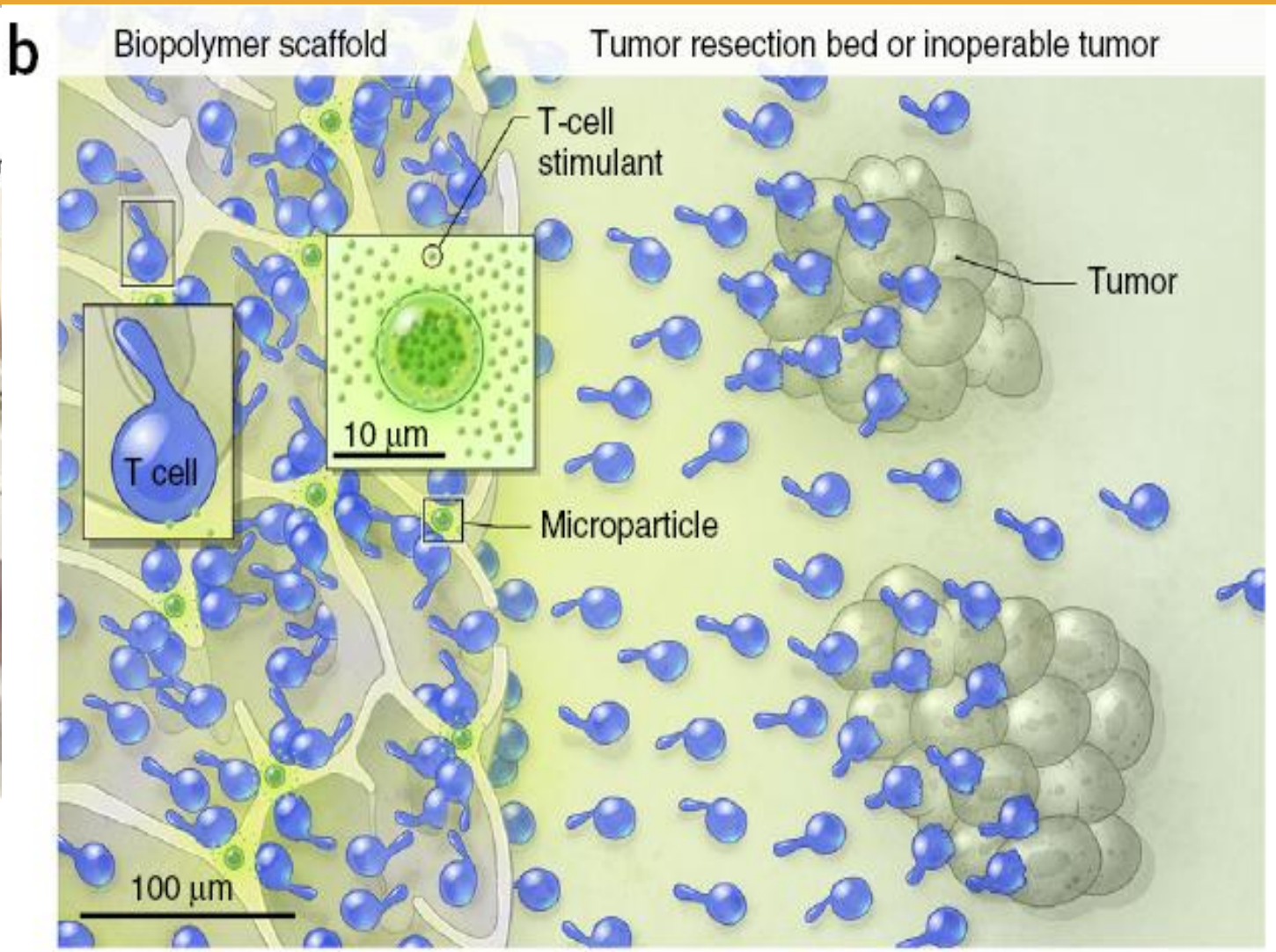
Fluorescent



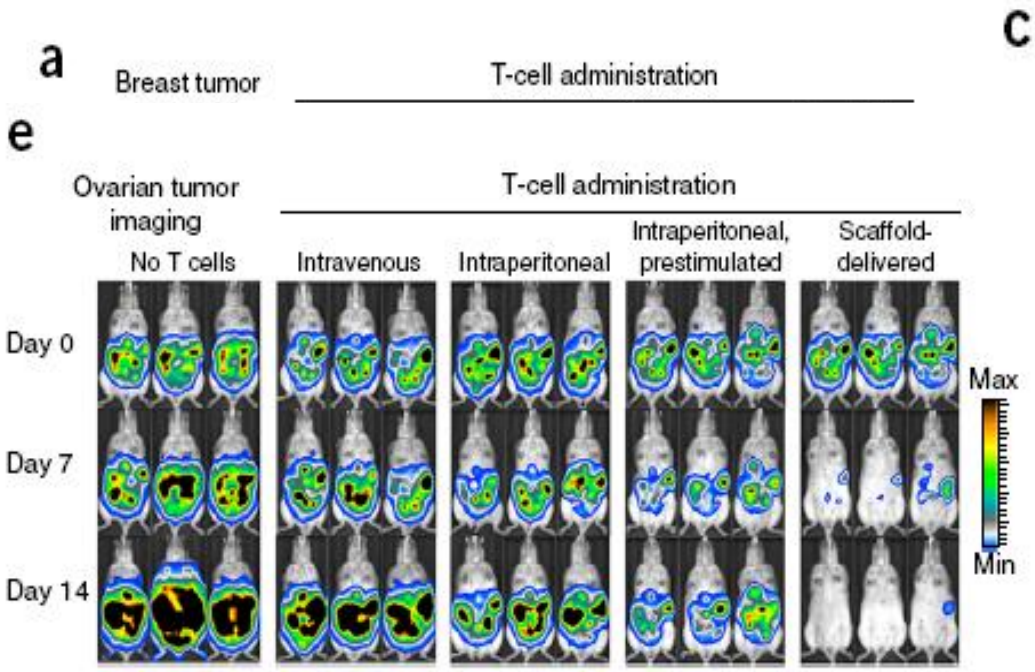
Isolation from transgenic mous

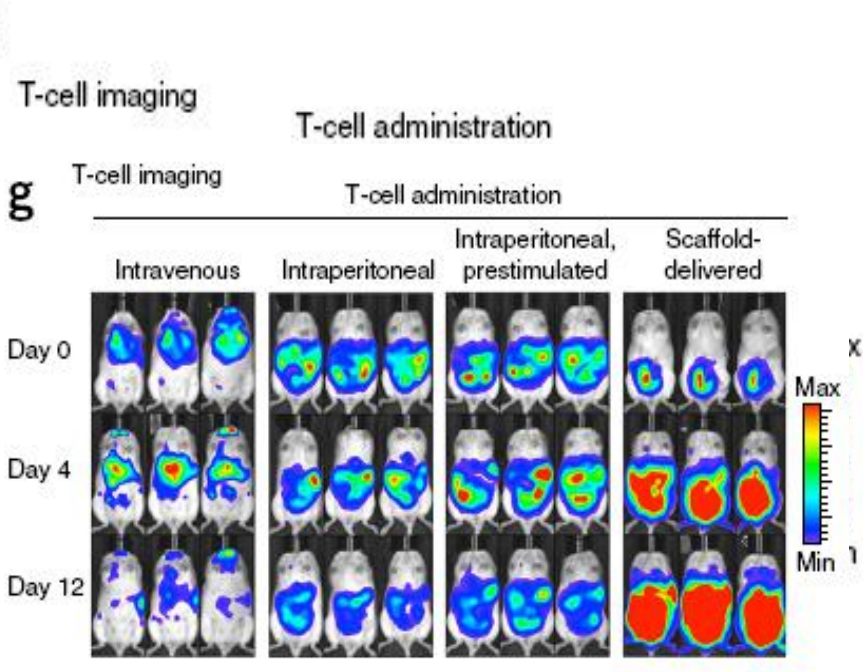


评价CAR-T的治疗效果和免疫反应



评价CAR-T的治疗效果和免疫反应





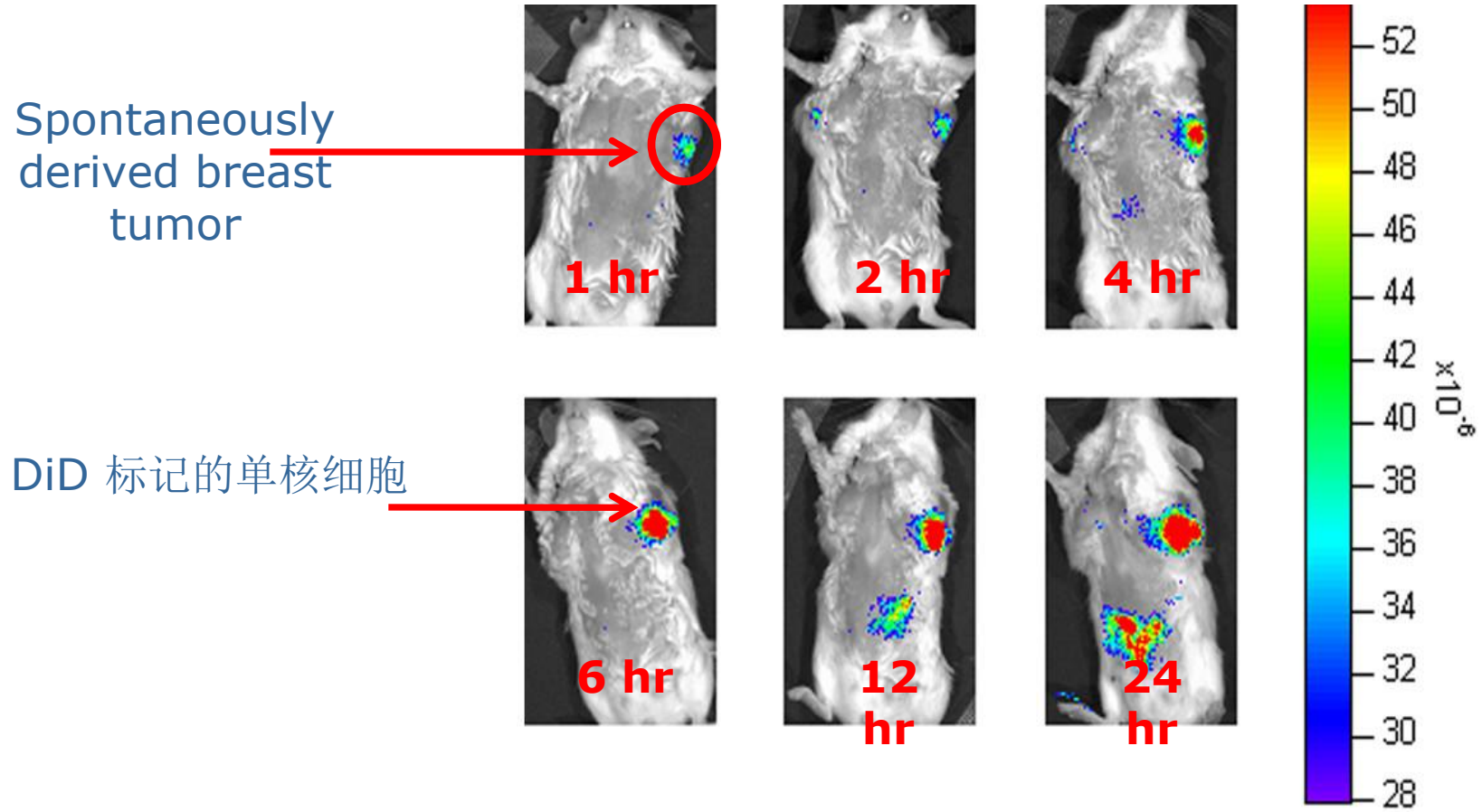
不同处理方式下肿瘤和T细胞的成像，建立小鼠ID8-VEGF-luc卵巢癌腹腔转移模型，为了获得卵巢癌特异性（NKG2D CAR-transduced）T细胞，取C57BL/6J小鼠的脾脏，过滤后重悬于裂解液中，分离的脾细胞使用含1 ng/ml interleukin-7和2 μg/ml Concavalin A的完全培养基RPMI1640培养，并纯化获得CD8+效应T细胞，CD8+效应T细胞先后转染NKG2D-CAR基因和CBR-luc。成像前分别注射F-luc底物和CBR-luc的底物进行肿瘤或T细胞的生物发光成像。

异性T
氧脱
像前

Sirkka B Stephan *et al.*, nature biotechnology.2015;33(1):97-104.

检测单核细胞向肿瘤的汇集

- MMTV-PymT 转基因鼠模型：良好的自发型乳腺癌模型
- 通过对单核细胞的招募评价肿瘤样的炎症反应

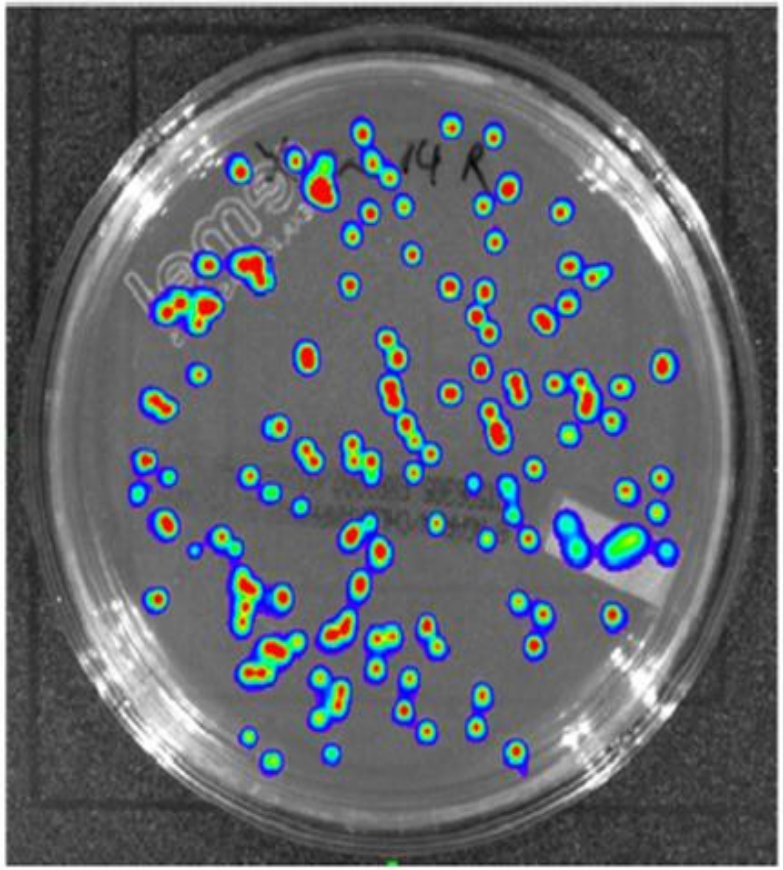


Sista et al, Journal of Translational Medicine (2009)

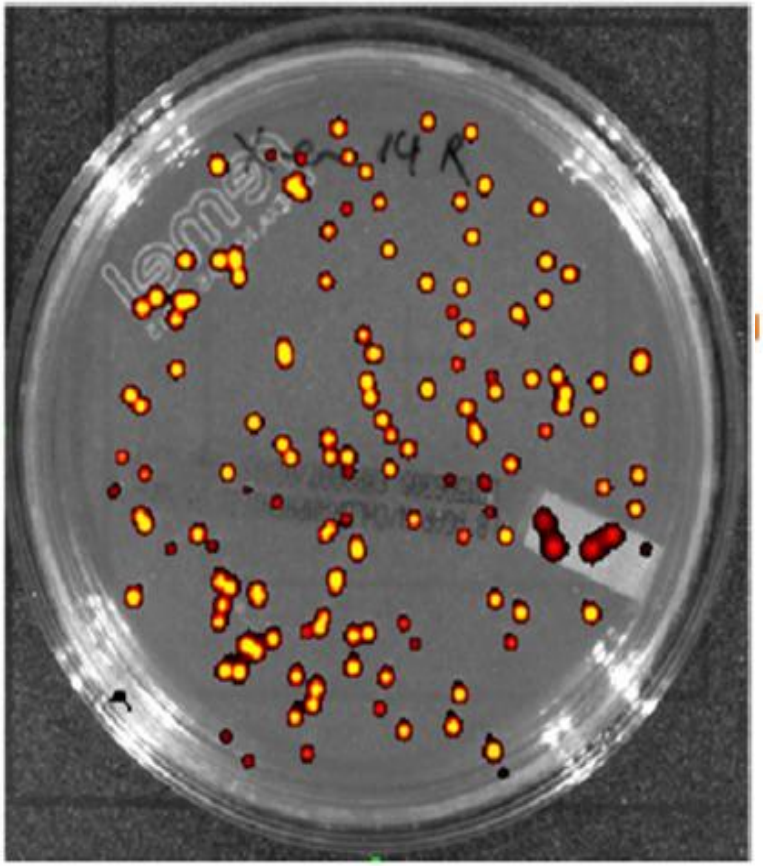
感染相关研究

应用举例——细菌、病毒的研究

BIOLUMINESCENCE

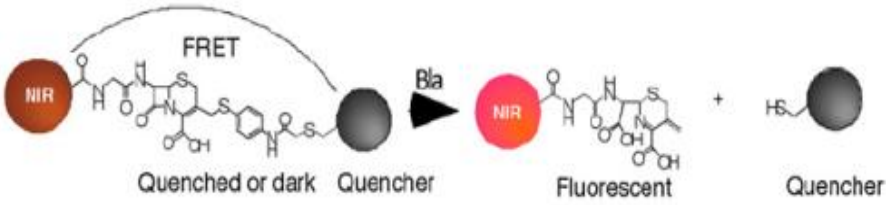


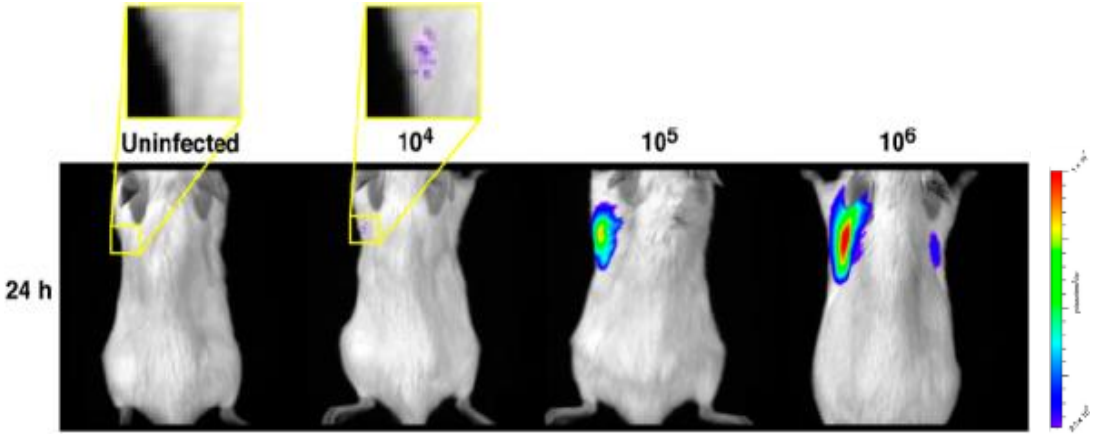
FLUORESCENCE

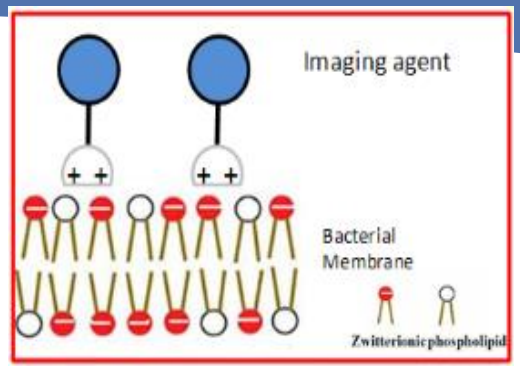


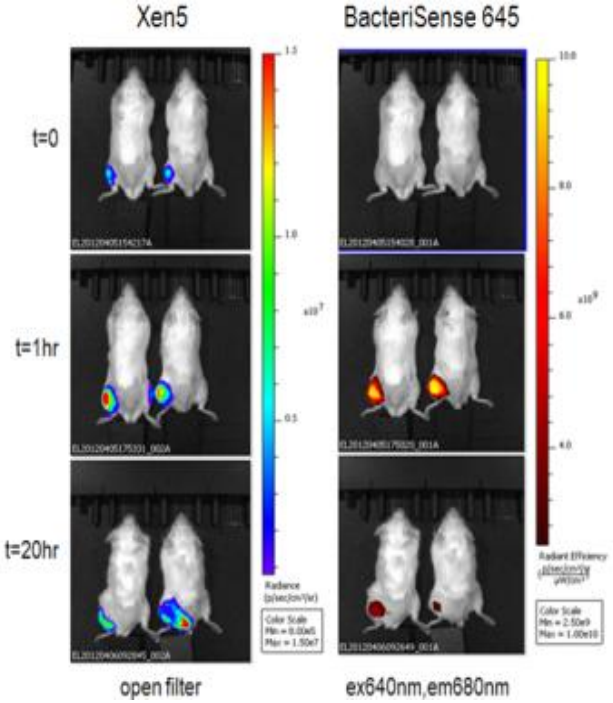
荧光探针检测分支杆菌

B内酰胺酶底物肽段







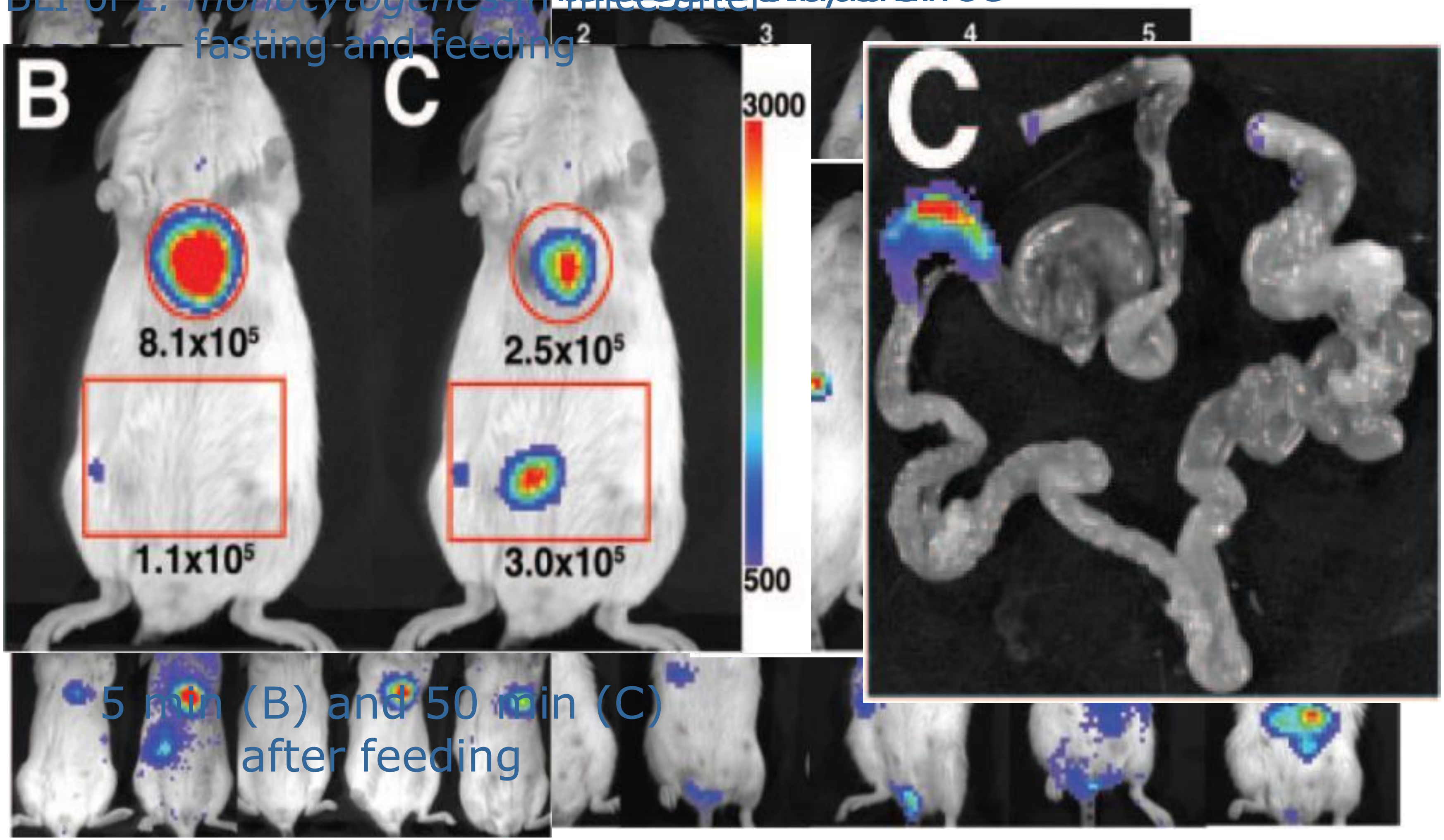


Kong et al, PNAS, 2010

活体监测单核细胞增多性李斯特菌感染的时空分布

Extended spatiotemporal distribution of *L. monocytogenes* in infected Balb/c mice

BLI of *L. monocytogenes* in mice after fasting and feeding

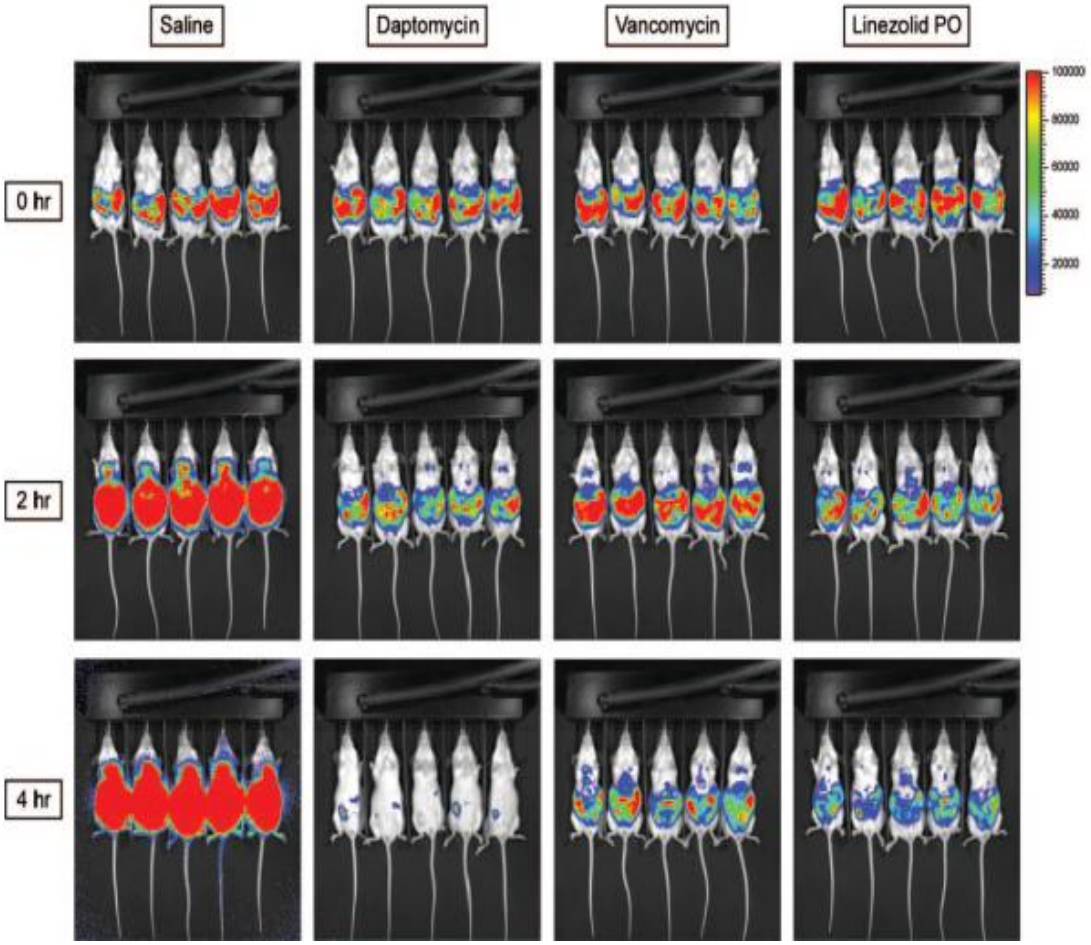


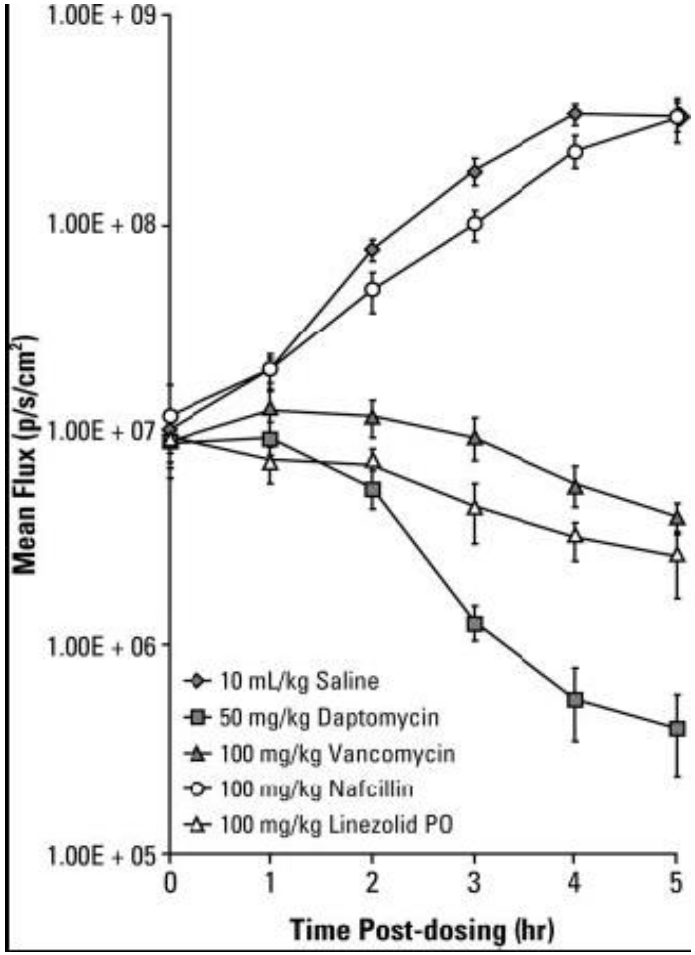
5 min (B) and 50 min (C) after feeding

Hardy et al, Science, 2004 (& 2006)

FDA-批准药物临床前评价

Cubicin (Daptomycin) is an FDA approved drug for infections caused by *Staphylococcus aureus*

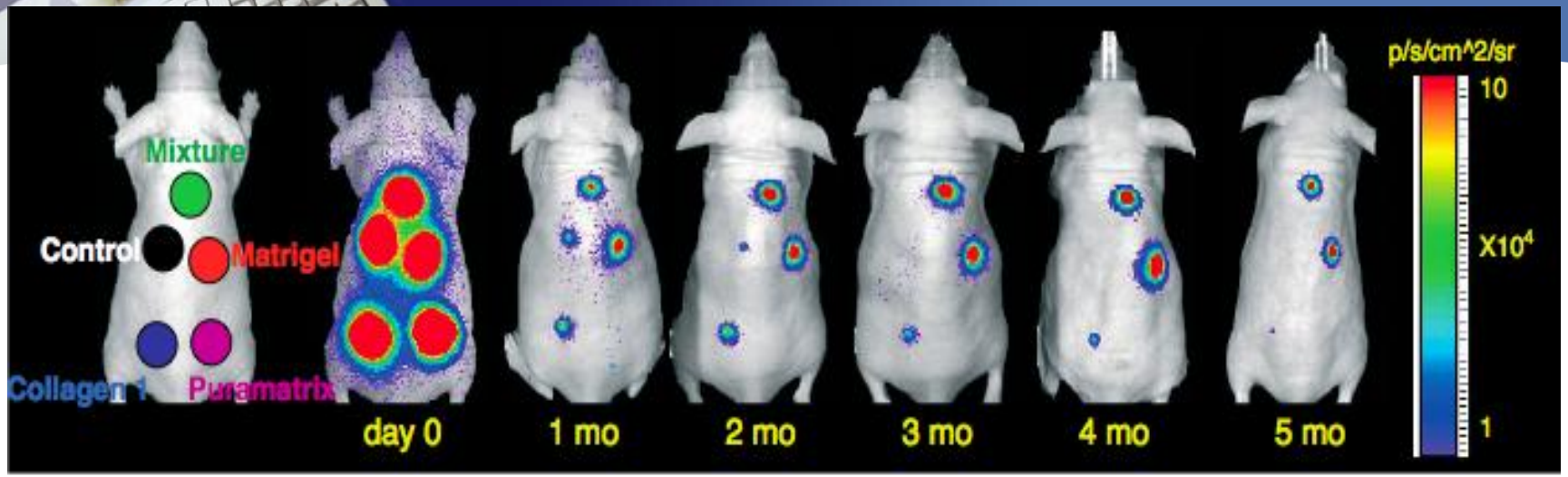


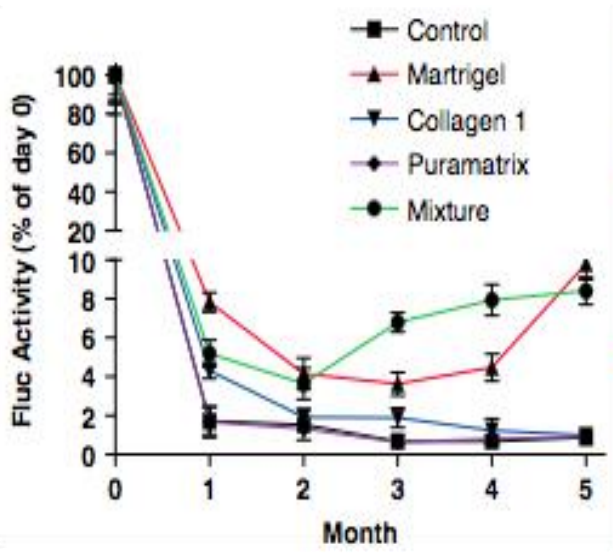


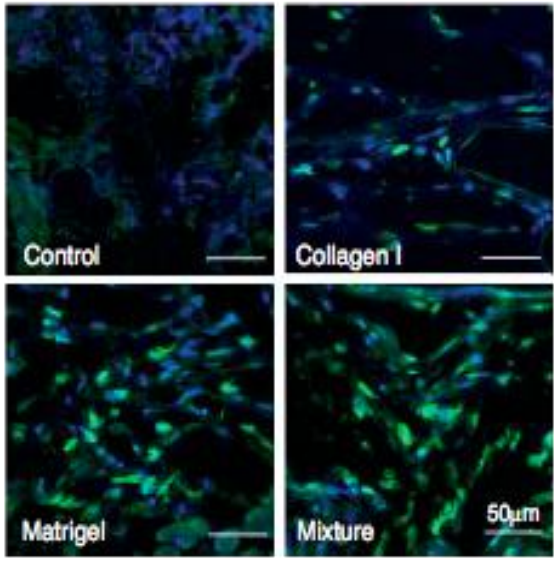
Mortin et al., Antimicrobial Agents and Chemotherapy,

干细胞相关研究

应用举例——评价干细胞的存活



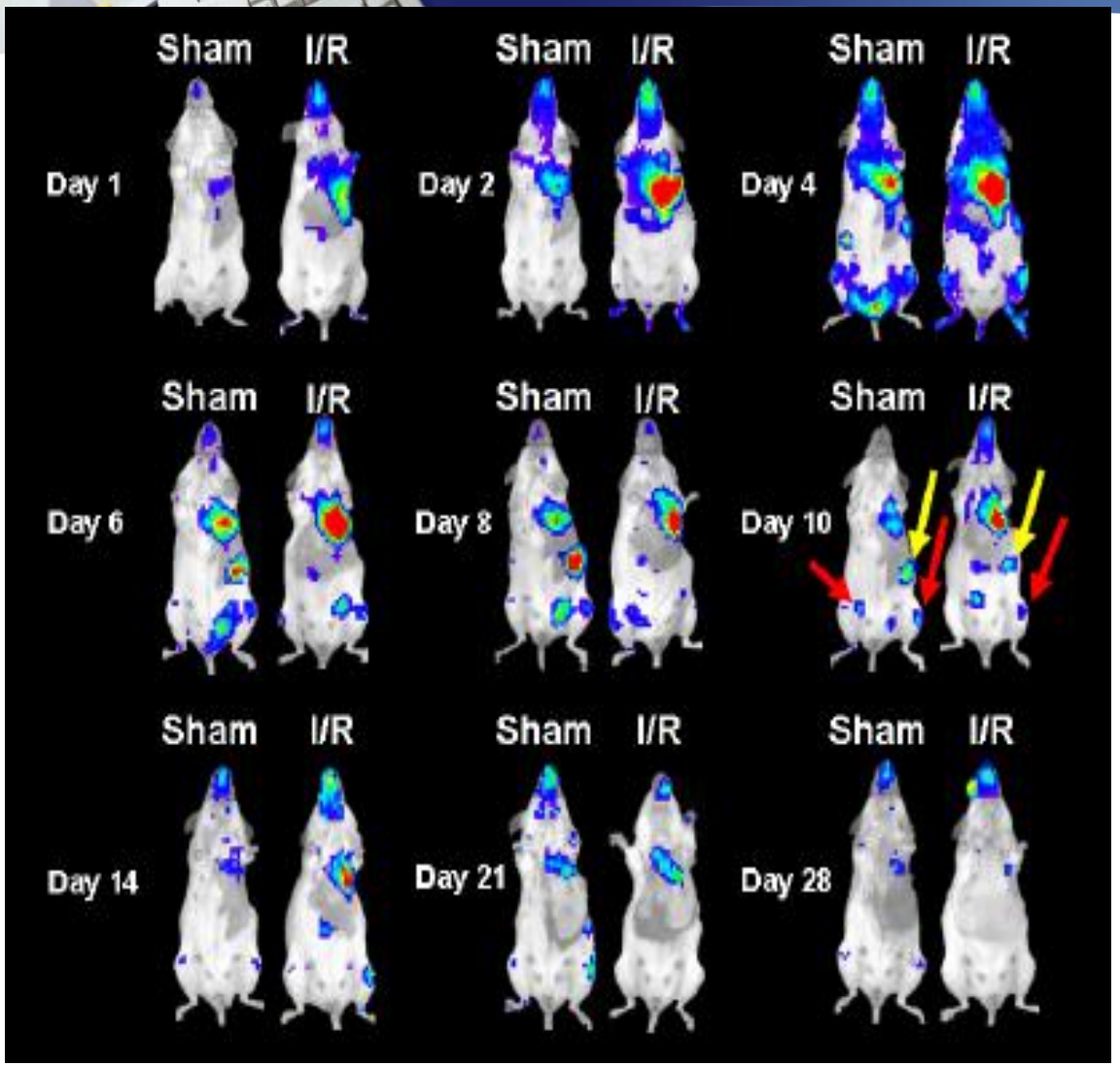




5 × 10^5 MSCsFluc+/eGFP

Cao *et al*, J Tissue Eng Reg Med, 20

应用举例——BMMC向局部缺血心脏的归巢

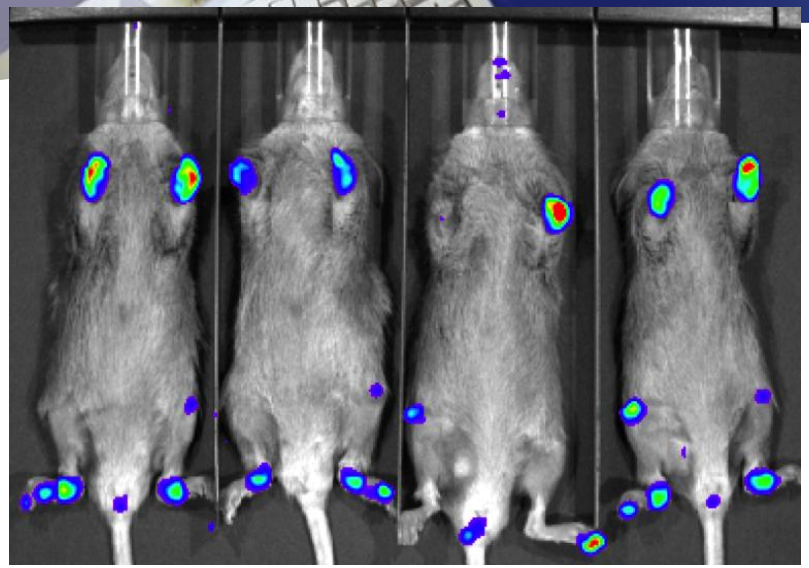


- Implanted bone marrow mononuclear cells (BMMC) preferentially home to hearts with ischemia re-perfusion injury (I/R), as compared to sham hearts;
- Echocardiography revealed a trend towards improved cardiac function in animals that received BMMC compared to PBS controls, but was not statistically significant.

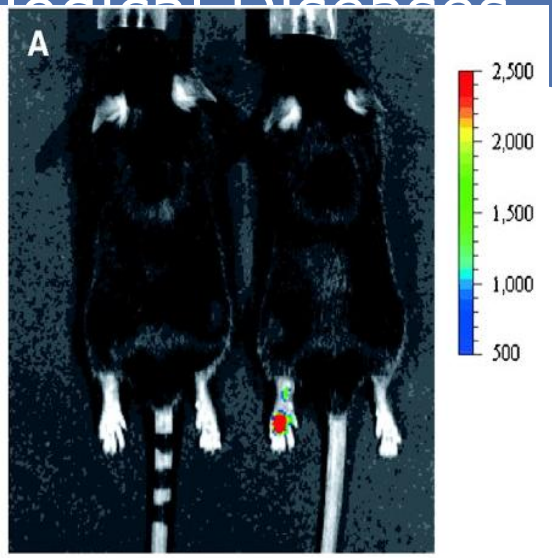
Sheikh et al, Stem Cells, 2007

免疫炎症研究

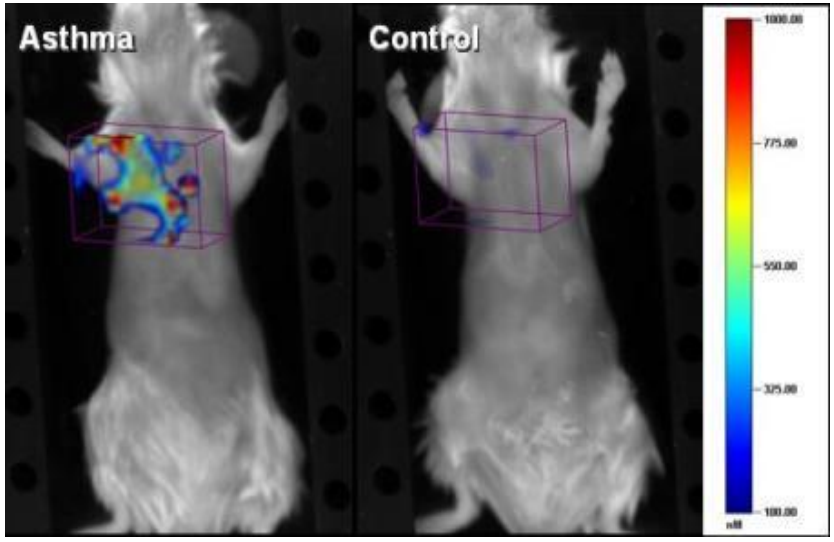
Using Functional Probe to Monitoring Immunological Diseases



RediJect Fluorescent COX-2 Probe to detect Cyclooxygenase -2 in Inflammation

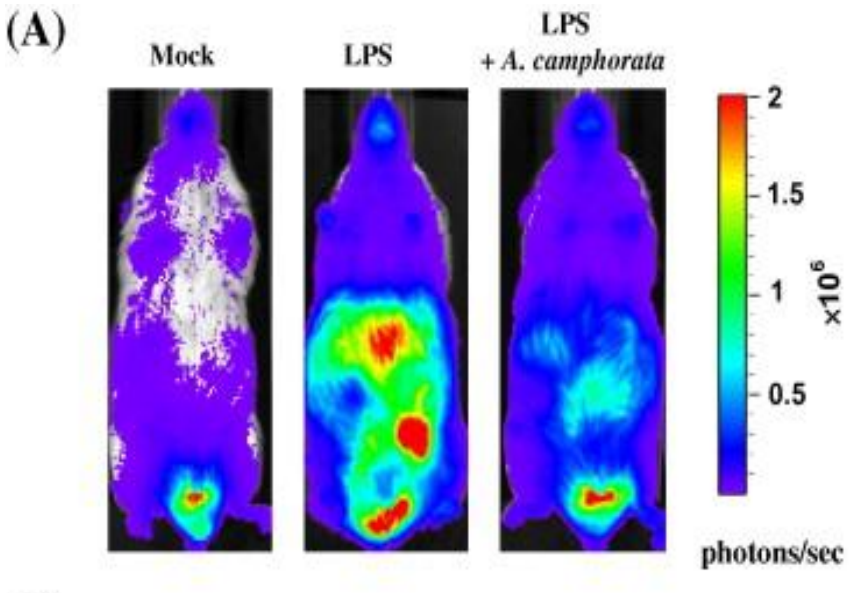


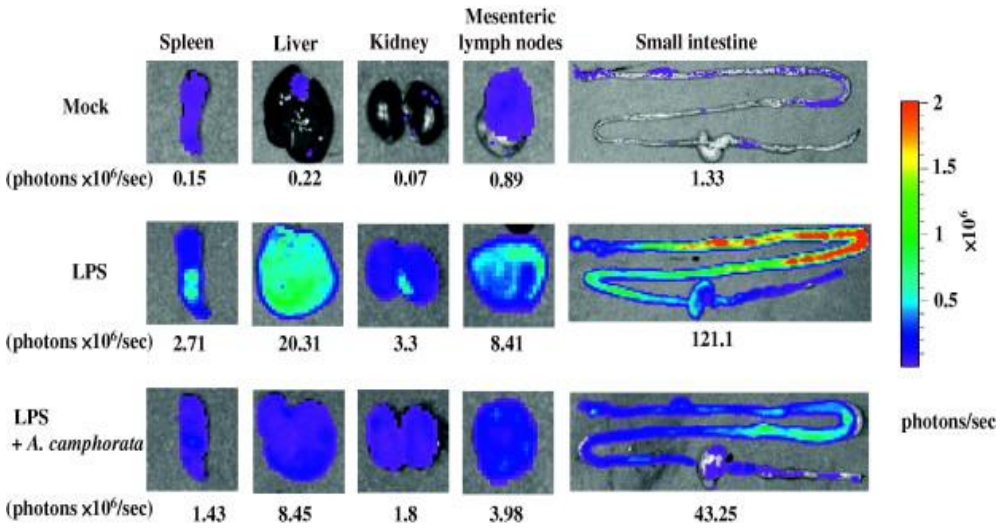
Chemiluminescent inflammation probe to detect myeloperoxidase (MPO) activity of activated phagocytes

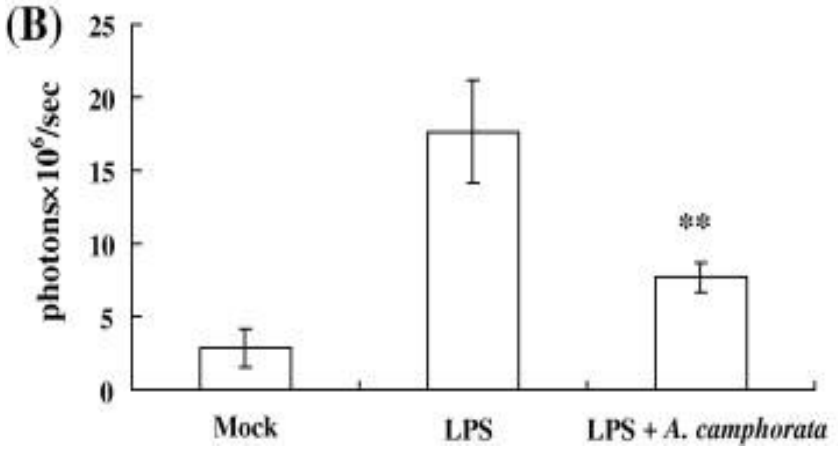


Activetable Fluorescent probe to detect Protease activity in Inflammation

评价保健品牛樟芝预防治疗炎症效果







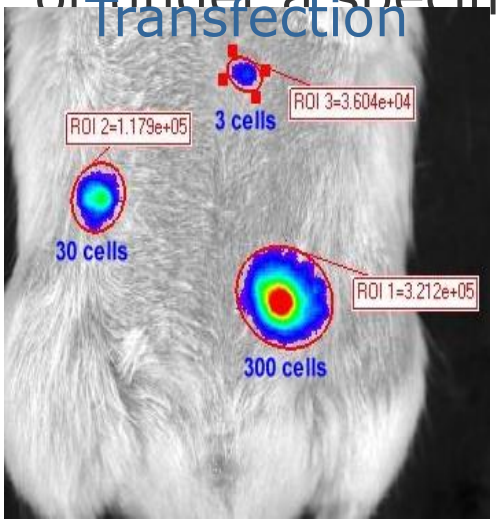
- ***Antrodia Camphorata*** is a unique mushroom from Taiwan that is said to have medicinal properties
- This study highlights the ability of *A. Camphorata to* inhibit LPS-induced inflammation in NFκB-luc mice, in vivo (left) and ex vivo (right)

Hseu *et al*, Food and Chemical Toxicity (2010)

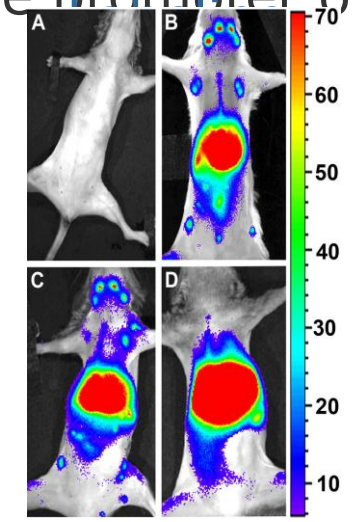
免疫细胞标记方法

- Stable transduction of murine and human immune cell populations with viral vectors carrying a luciferase/fluorescent reporter gene
- Short-term labeling with fluorescent dyes (e.g. DiR, DiD etc.)
- Isolation of immune cells from a transgenic mouse/rat expressing luciferase under the β-actin promoter (ubiquitous) or under a specific gene promoter of interest (inducible)

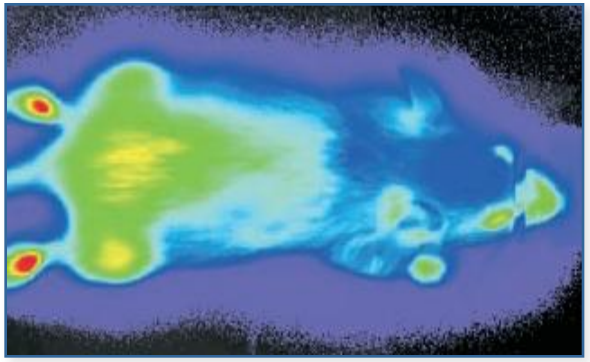
Stable Transfection



Fluorescent

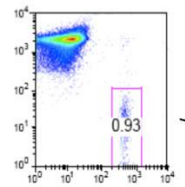


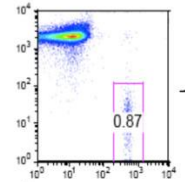
Isolation from transgenic mous

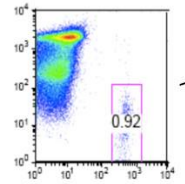


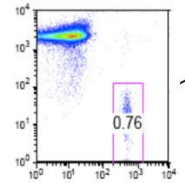
Transgenic Mouse Model for Studies of T-Cell Dynamics

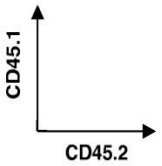
A novel transgenic mouse model relying on the human CD2 mini-gene to direct luciferase expression specifically to the T- cell compartment; Applications in immunotherapy or vaccine design



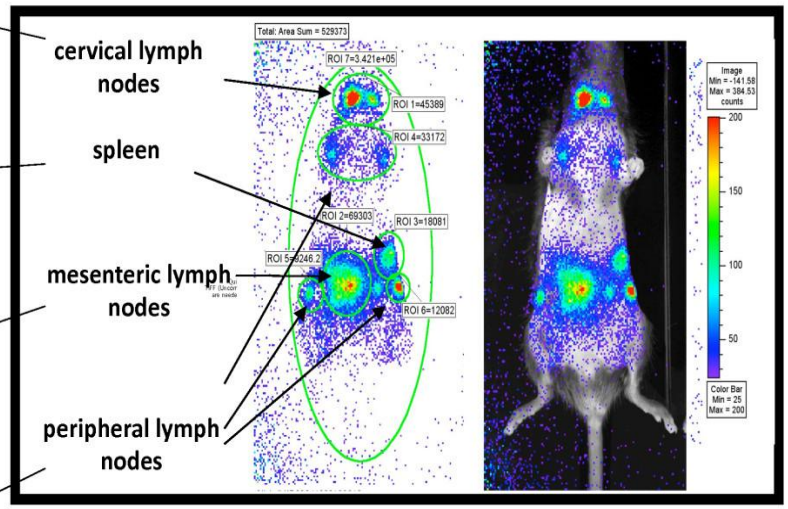




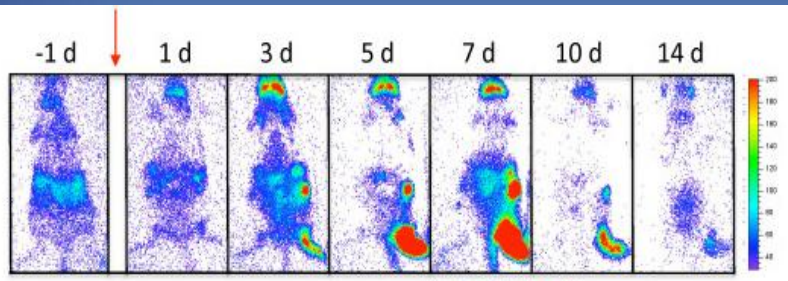


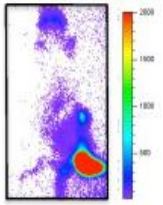


Three days post-transfer

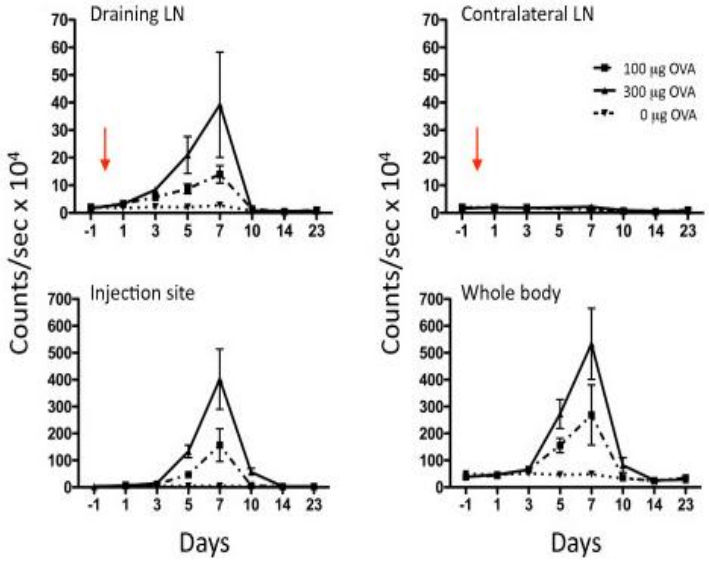


Biodistribution of adoptive transferred CD3⁺ T-cells within different lymphoid tissues



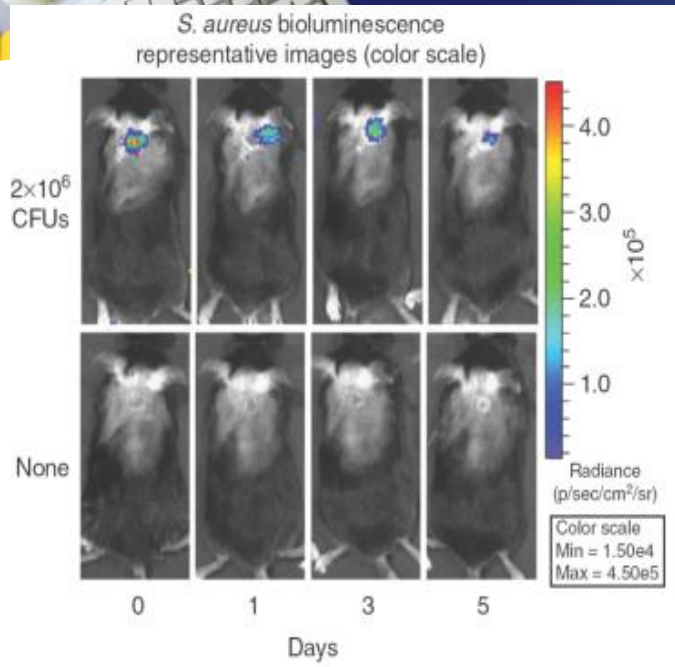


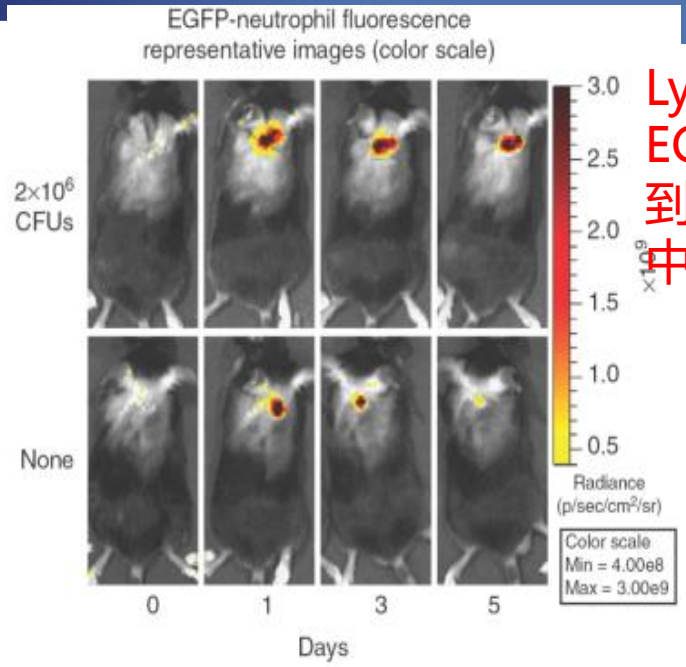
Clonal T-cell Expansion in response to antigen



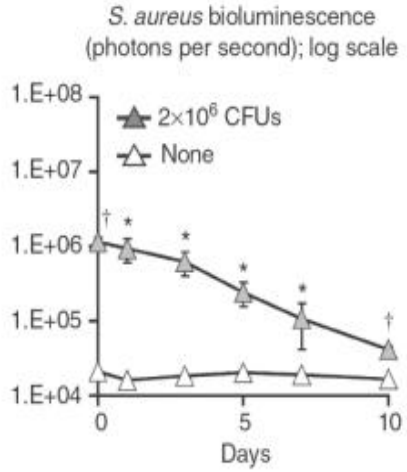
Chewning *et al*, BMC Immunology (2009)

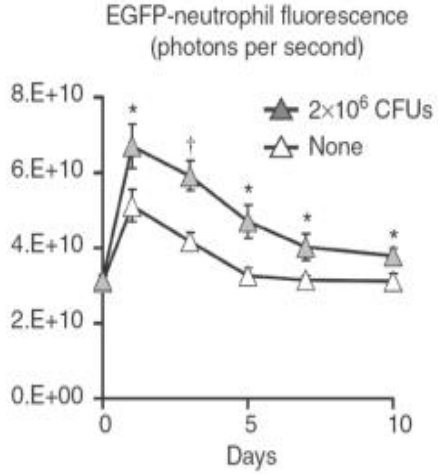
皮肤感染*S.aureus*后宿主免疫反应





LysEGFP mice：EGFP的基因插入到溶菌素的基因中。



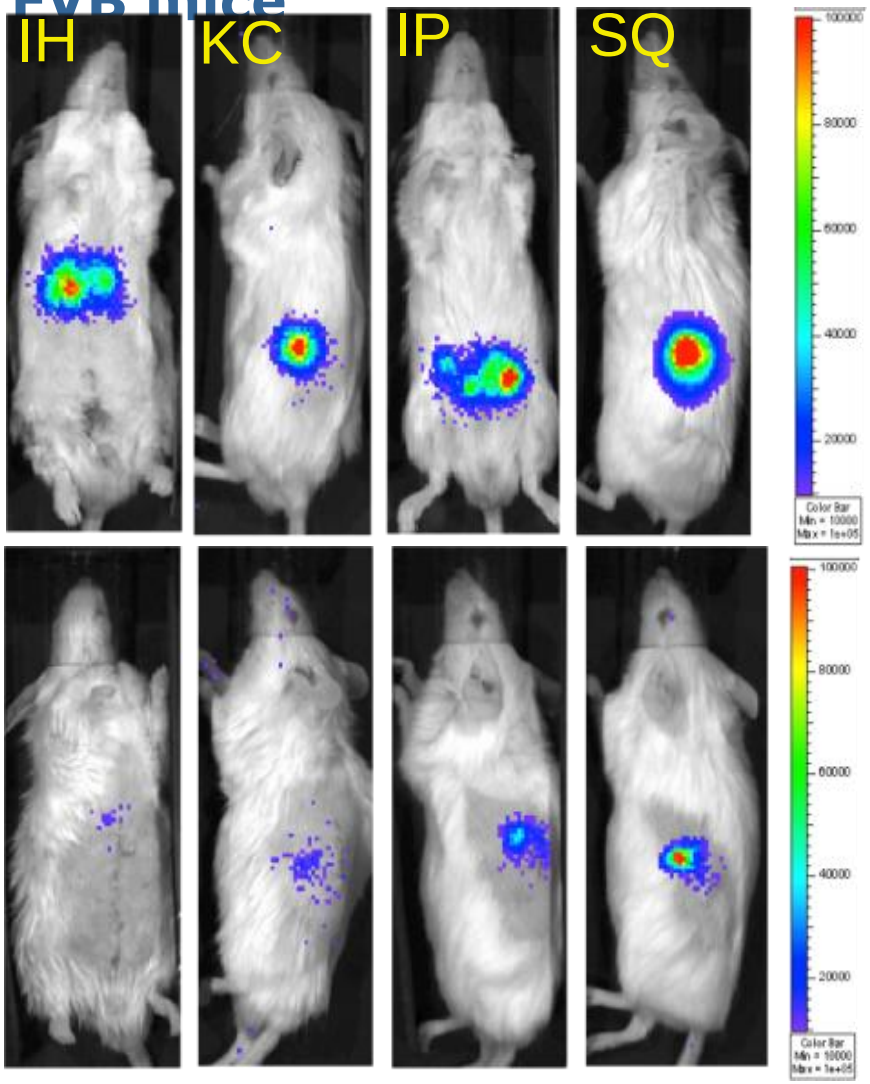


Cho *et al*, J. Invest. Dermatology (2011)

代谢相关疾病研究

应用举例——胰岛移植研究

Pancreatic Islets from FVB-Tg(RIP-luc) Mouse Transplanted into Syngeneic FVB mice

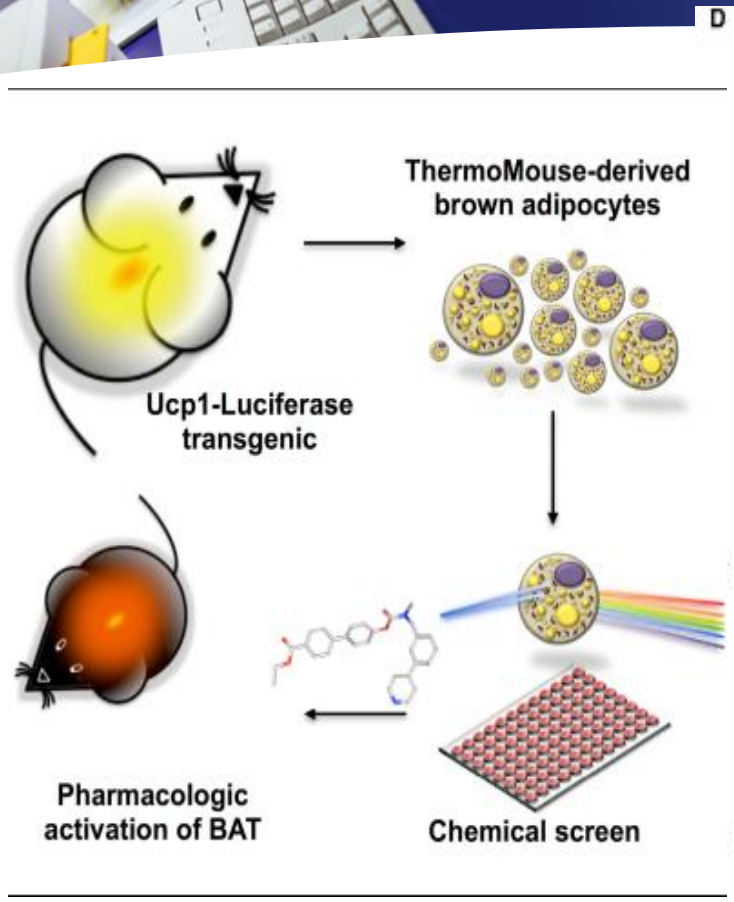


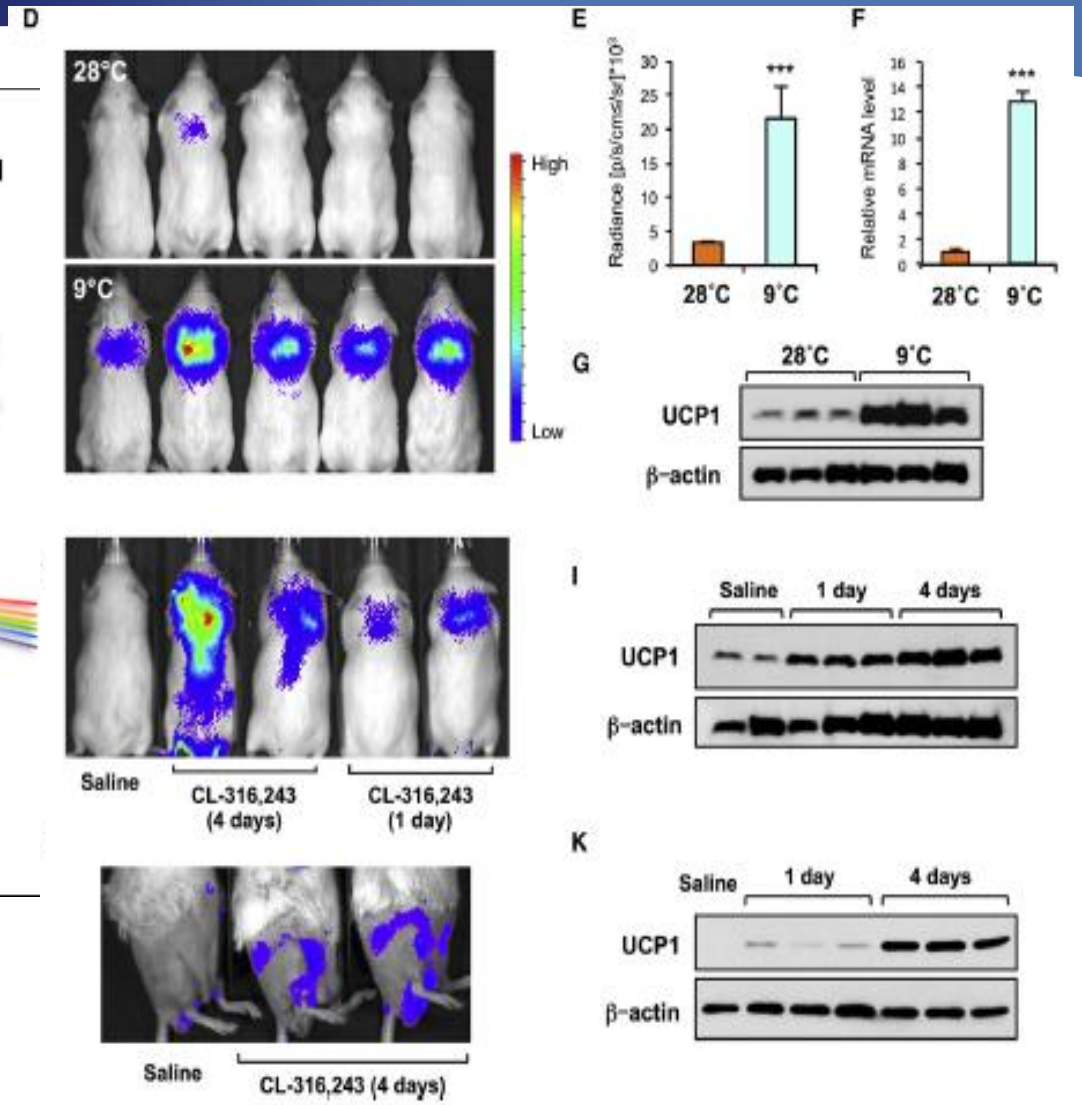
150 islets

10 islets

Chen et al, Transplantation, 2006

应用举例——研究棕色脂肪的活性





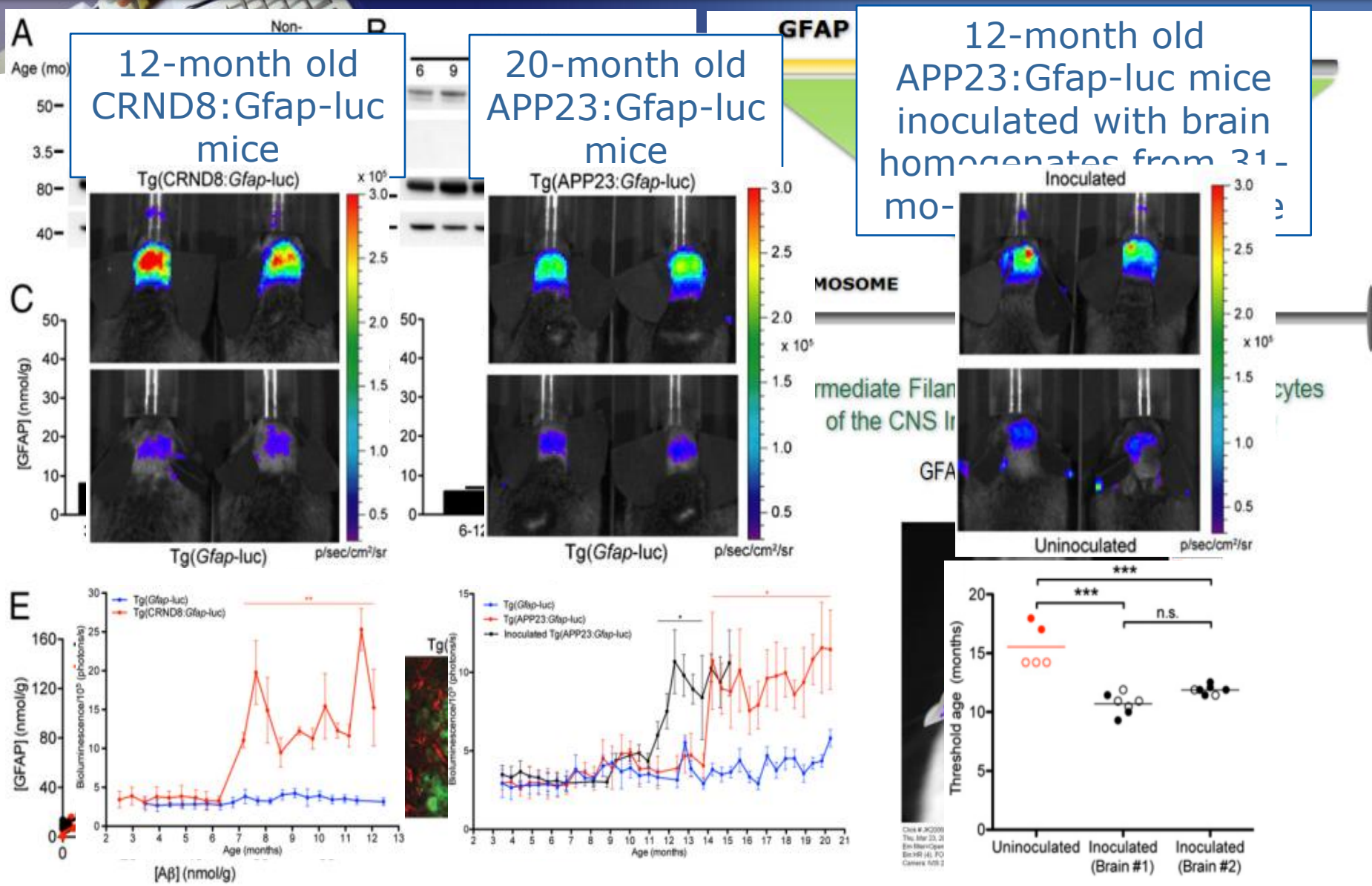
神经系统疾病研究

应用举例——BLI 检测小鼠阿尔茨海默模型中Aβ 淀粉样蛋白沉积

12-month old CRND8:Gfap-luc mice

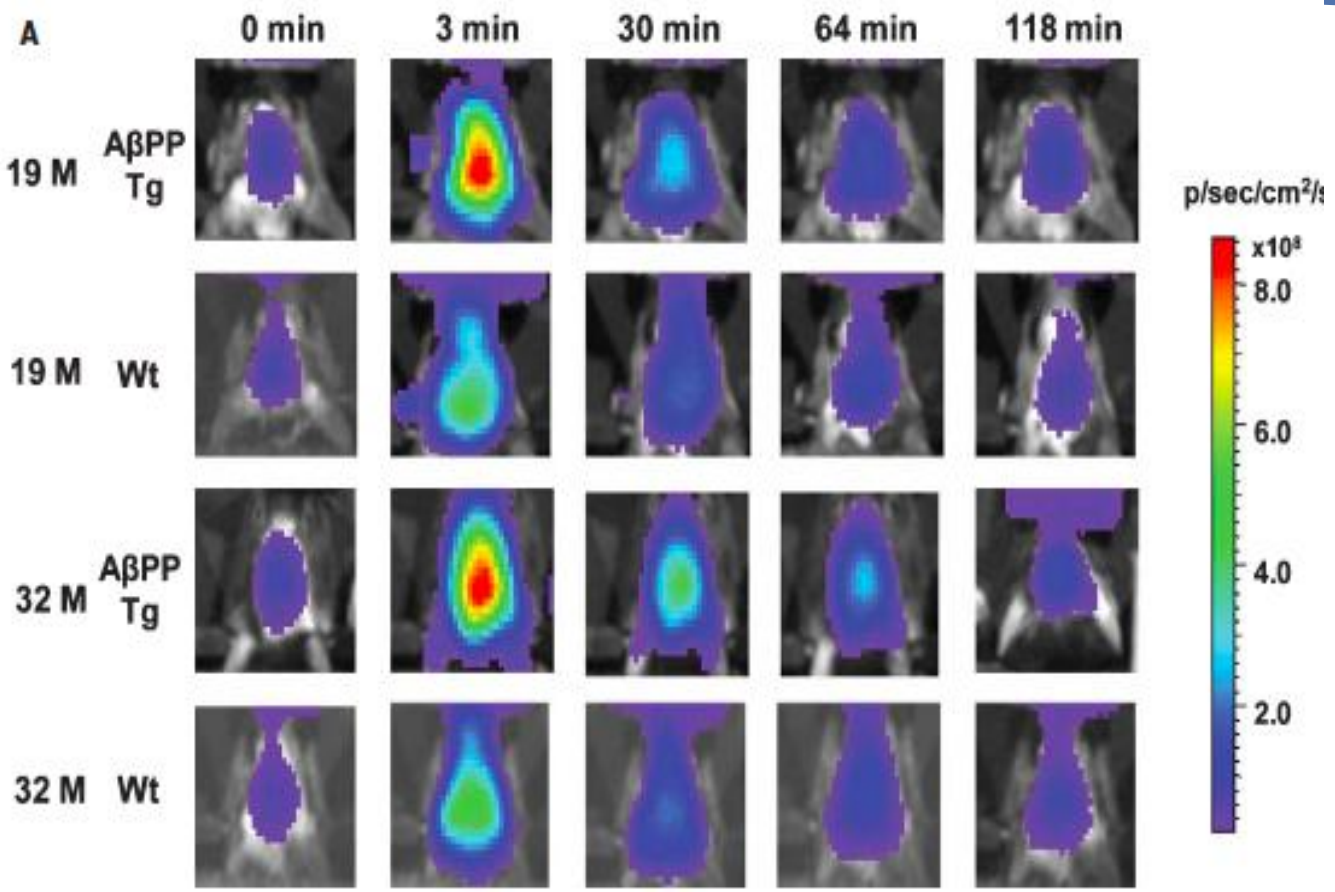
20-month old APP23:Gfap-luc mice

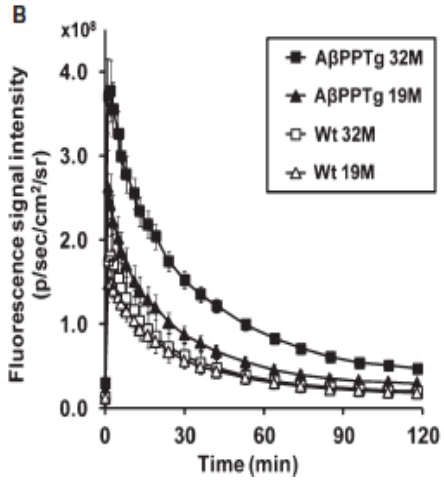
12-month old APP23:Gfap-luc mice inoculated with brain homogenates from 31-mo-

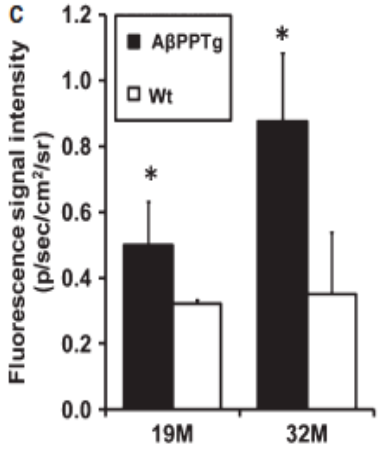


Watts, Prusiner *et al*, PNAS, 2011

应用举例——NIRF 检测小鼠脑部淀粉样斑块



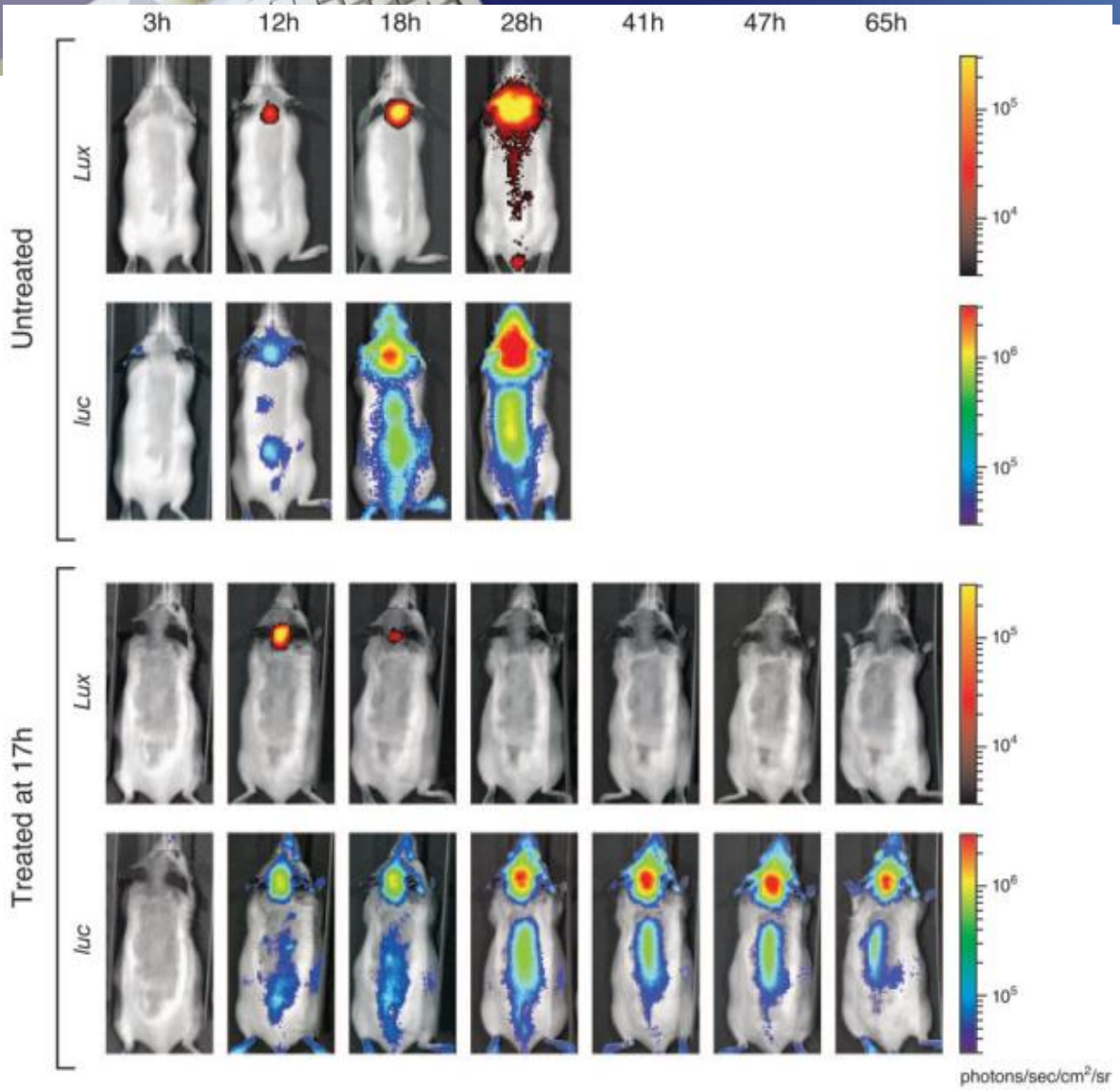




Nice correlation between the fluorescence intensity of their THK-265 NIRF probe (excitation 630 nm; emission 670 nm) and amyloid plaque burden in the brains of APP transgenic mice

Okamura *et al.* J Alzheimer's Disease (2011)

应用举例——细菌感染造成的神经系统损伤



- Measure infection and host response simultaneously
- BLI allows monitoring two different bioluminescent reporters at the same time
- GFAP expressed even after bacterial infection treated
- Study showed pneumococcal infection causes neuronal damage

Thank You !