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HUMAN HEALTH

IN VIVO IMAGING

IVIS®小动物活体成像原理与应用

PE活体影像专家　黄幸

Xing.Huang@perkinelmer.com

13051590380

分子影像技术概览

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活体光学成像——技术核心

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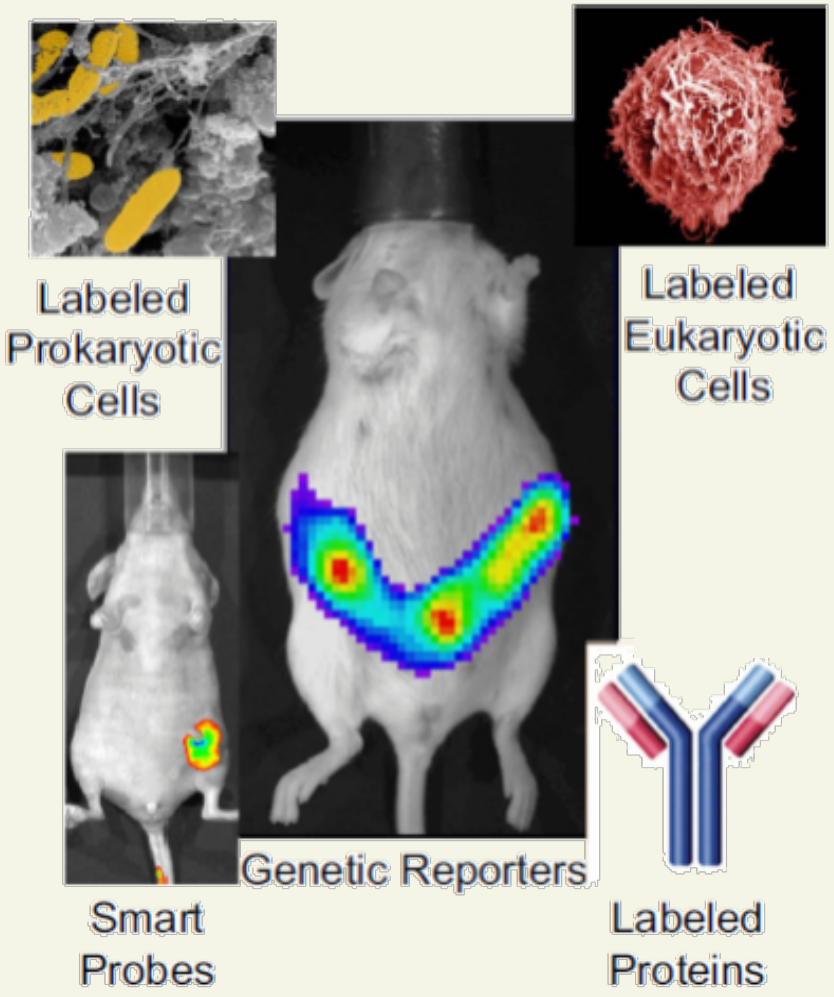
小动物活体光学成像——是通过一定的方式对研究对象进行光学标记，使其具有发光的性质，再通过成像技术及设备对光信号进行采集成像。

光学标记



成像技术及设备

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活体光学成像——标记方式

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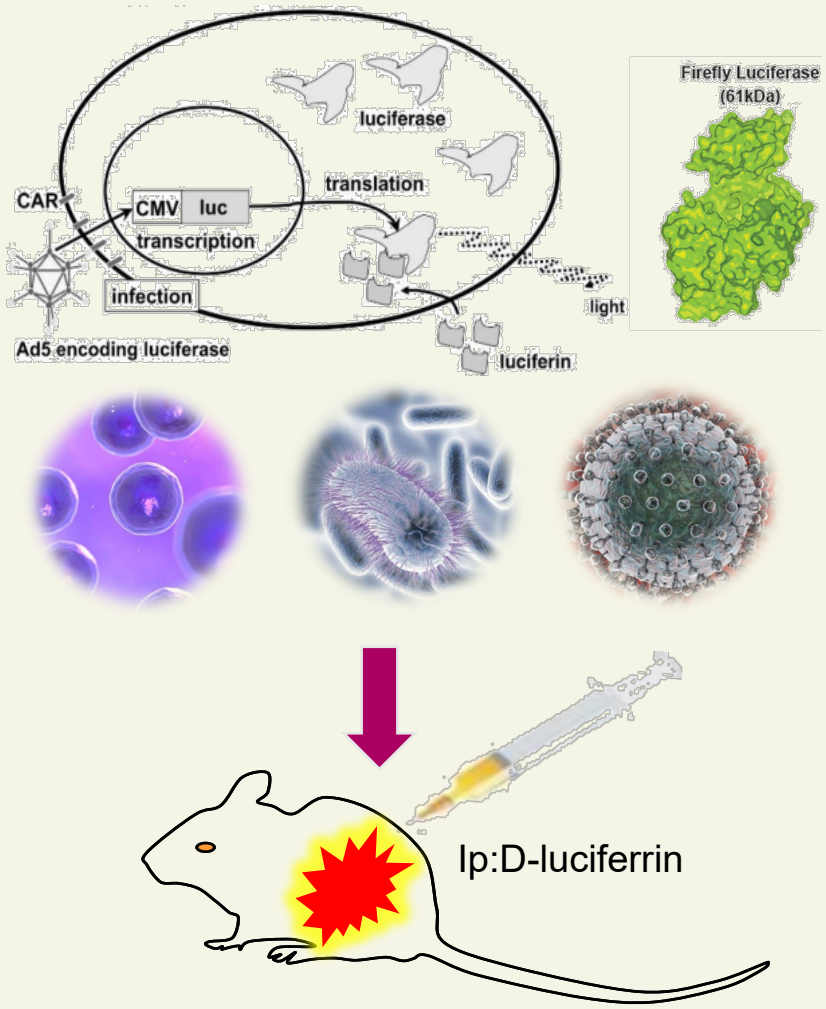
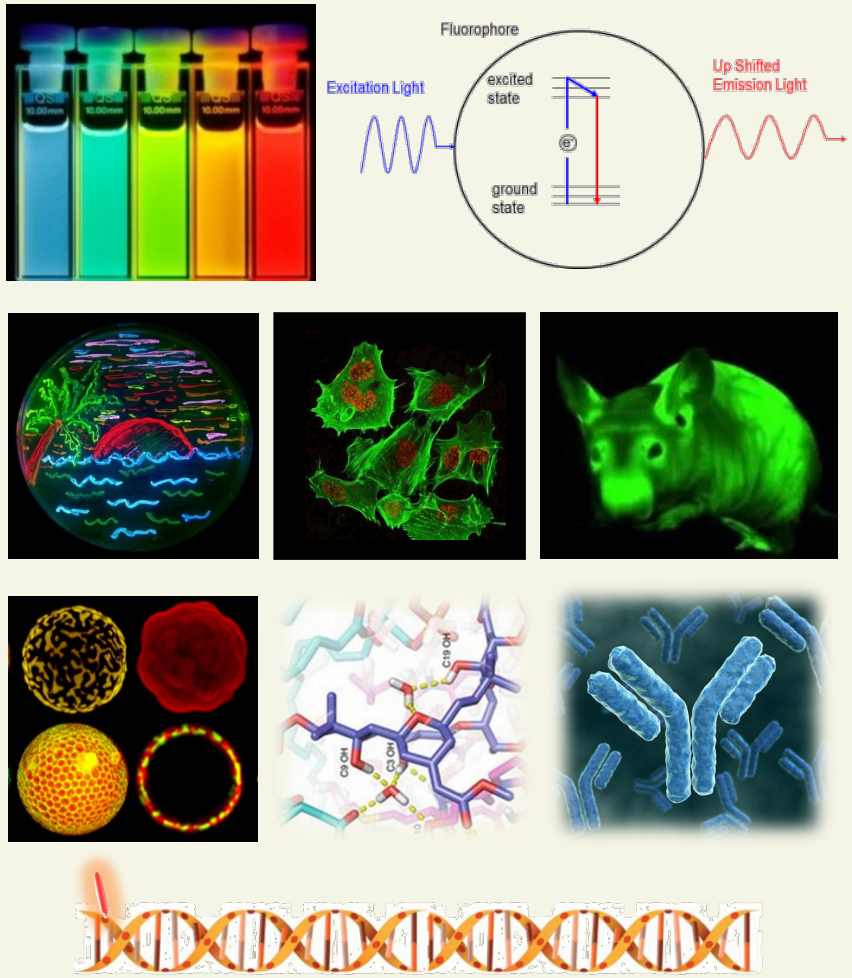
Bioluminescence



Fluorescence



荧光报告基因表达

荧光染料探针标记

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常用荧光素酶及分类

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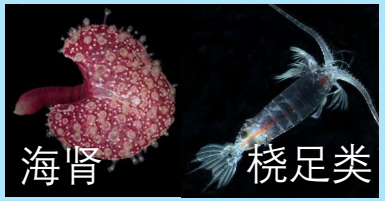
Table 1. Luciferase Reporters Commonly Used for *In Vivo* BLI and Their Specifications

Bioluminescent reporter	Emission peak *in vivo*	Size (kDa)	Substrate	Cofactors	Codon optimized for mammalian expression	Refs
Photinus pyralis; North American firefly luciferase	~600 nM	62	D-Luciferin	O_2, Mg^{++}, ATP	YES (Luc2)	[7,8]
Photinus pyralis red; North American firefly luciferase	618 nM	62	D-Luciferin	O_2, Mg^{++}, ATP	YES (PpyRE8/9)	[9]
Luciola italica red; Italian firefly luciferase	~620 nM	64	D-Luciferin	O_2, Mg^{++}, ATP	YES (L.it RE)	[10]
Pyrophorus plagiophthalamus; click beetle luciferase	Red: 613 nM	64	D-Luciferin	O_2, Mg^{++}, ATP	YES (CBred)	[11]
	Green: 537 nm				YES (CBG99, CBG68)	[7,11]
Renilla reniformis; sea pansy luciferase	hRLUc 480 nM	36	Coelenterazine	N/A	YES (hRLuc)	[7,12]
	hRluc8 480 nM	36	Coelenterazine	N/A	YES	[13,14]
	hRluc8.6 535 nM	36	Coelenterazine	N/A	YES	[13,14]
Gaussia princeps; *Gaussia* luciferase	480 nM	20	Coelenterazine	N/A	YES (hGluc)	[15]
Oplophorus gracilirostris; NanoLuc luciferase	460 nM	19	Furimazine	N/A	YES (Nluc)	[16,17]
Cypridina noctiluca; *Cypridina* luciferase	~460 nM	62	Cyprinidid luciferin	N/A	YES (Cluc)	[18]
Vargula hilgendorfii; sea firefly luciferase	~460 nM	62	Cyprinidid luciferin	N/A	YES (Vluc)	[19,20]
Aequorea Victoria; aequorin photoprotein	~460 nM	62	Coelenterazine	O_2, Ca++	YES (opAEQ)	[21,22]
Photorhabdus luminescens; bacterial luciferase	490 nM	40 and 37	$FMNH_2$, long chain fatty aldehyde	O_2	YES (LuxAB)	[23]

底物为
D-荧光素

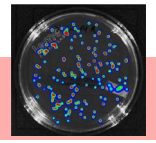


底物为腔肠素

海虾

海萤

底物为
海萤荧光素



改造的发光细菌

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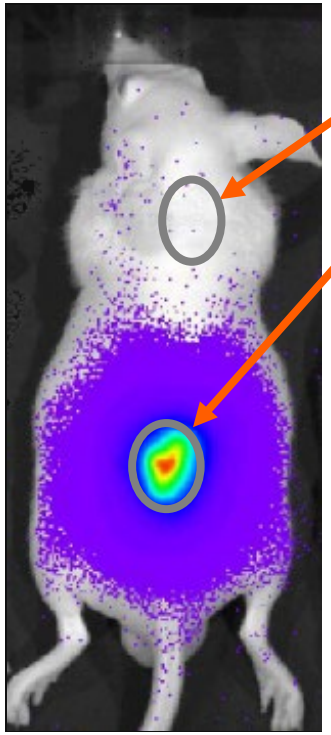
成像模式选择——生物发光 vs 荧光

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

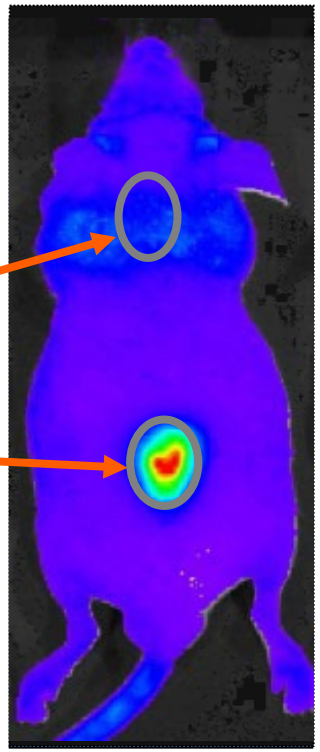
信噪比 ~ 7500

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.

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波长选择—透过率和背景噪音

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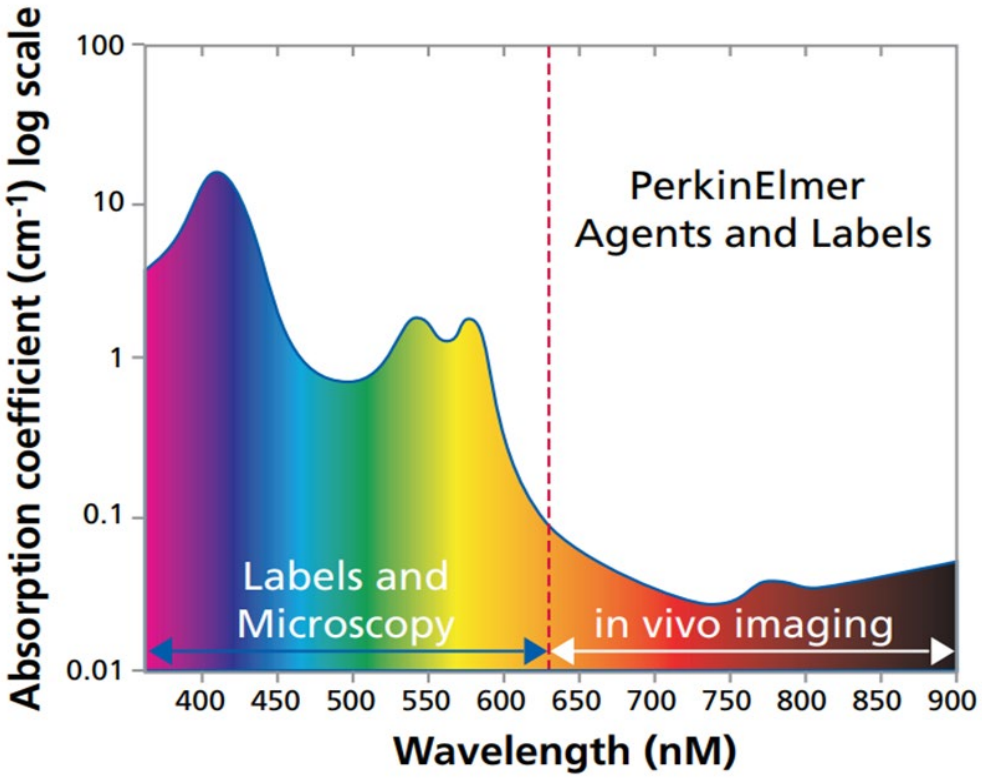
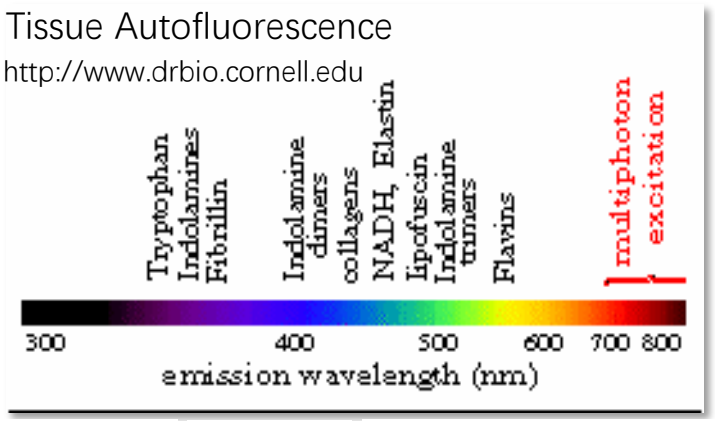
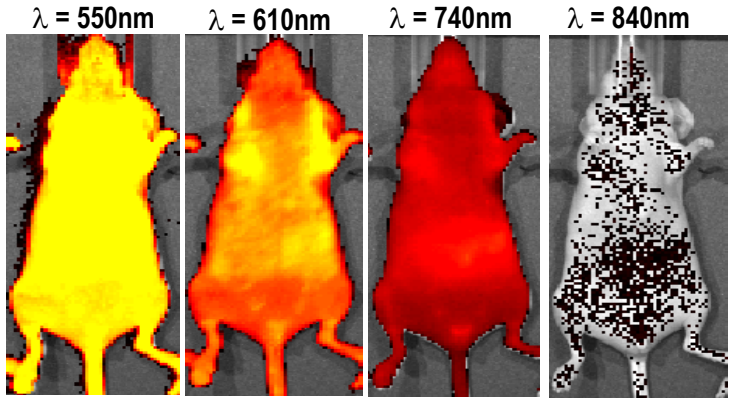
SNR= Signal / Autofluorescence

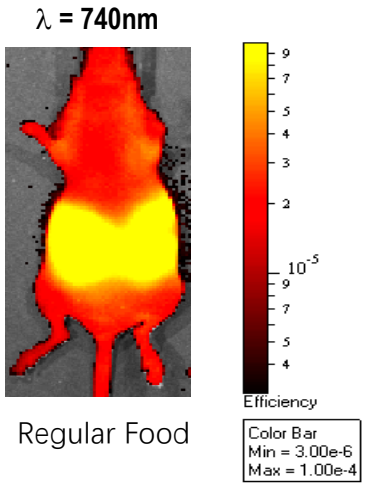


血红蛋白和其他机体组分强烈吸收可见光。
在近红外区域，组织吸收显著降低。





Alfalfa Free Rodent Food



Regular Food

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细胞生物发光效率测试

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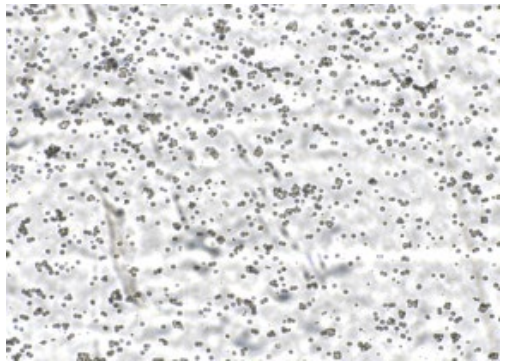
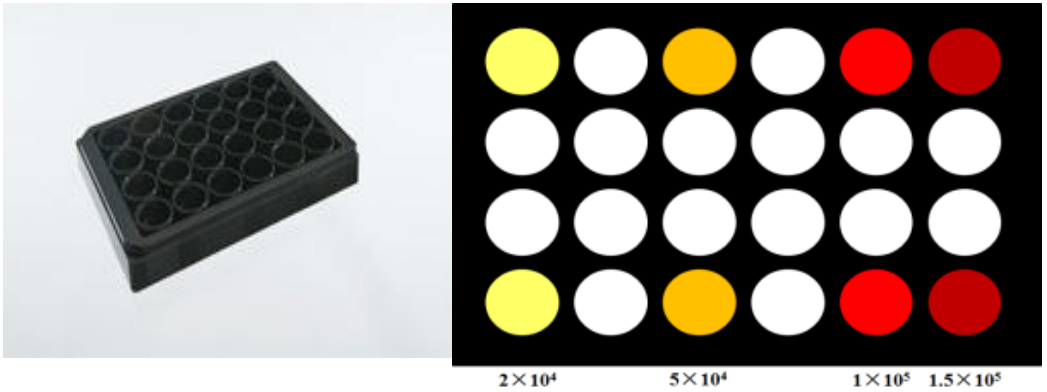
① 标记细胞的计数

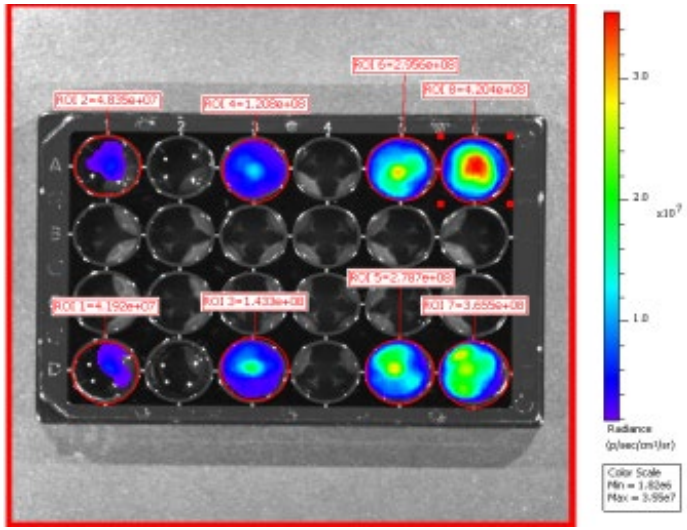


② 梯度细胞种板

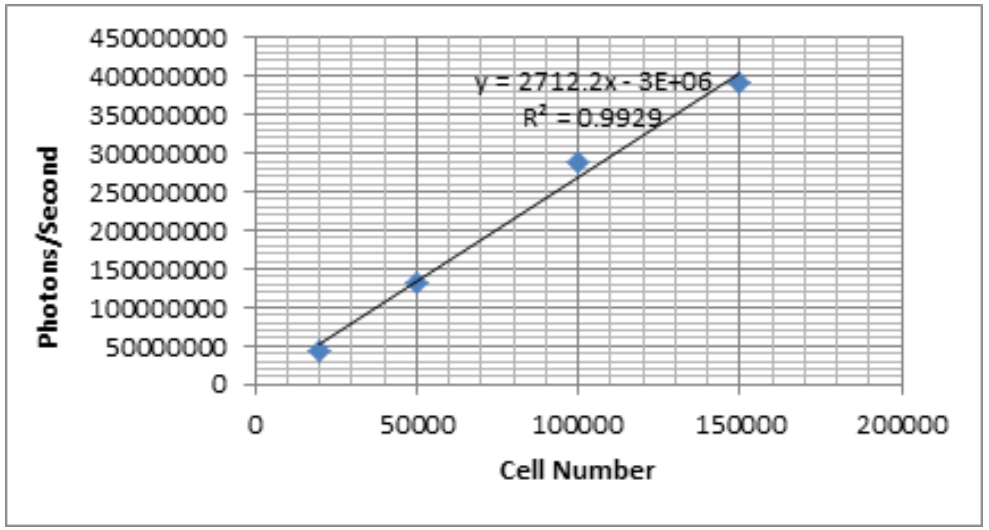


选择5个合适的梯度，进行对半稀释，实验中的工作细胞数可以设置在第三梯度

③ 生物发光成像



④ 标准曲线绘制，直线拟合



拟合的方程斜率值要

大于等于200

消化后的细胞用150 μg/ml D-luciferin底物进行重悬，2 min后使用IVIS系统成像，对孔板里的信号进行定量

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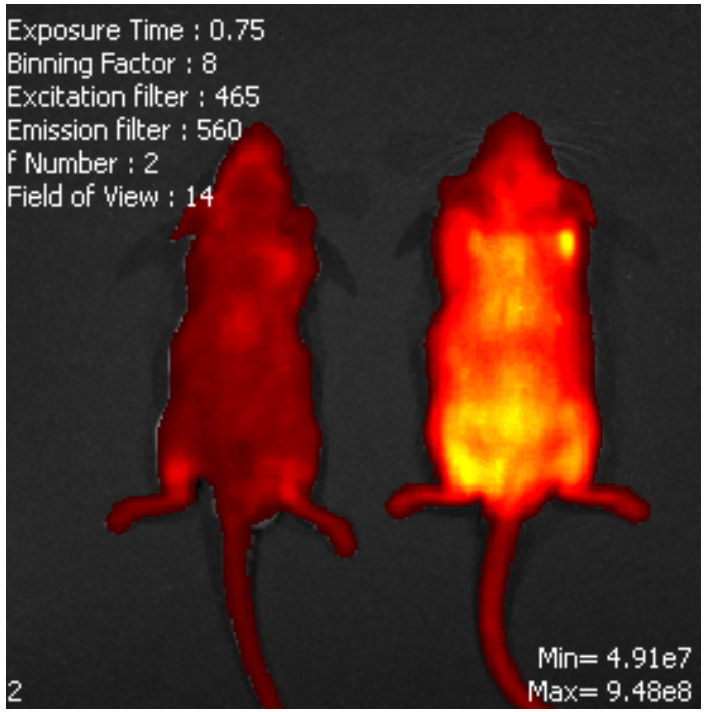
荧光成像小鼠剃毛——降低光吸收



Depilation not required

- Nude mice
- SKH1-E

Depilation required

- Normal, haired mouse strains (BALB/c, C57BL/6 etc . . .)

- 剃毛作用：降低毛发光吸收和光散射；去除来自毛发的背景荧光
- 实验前一天剃毛

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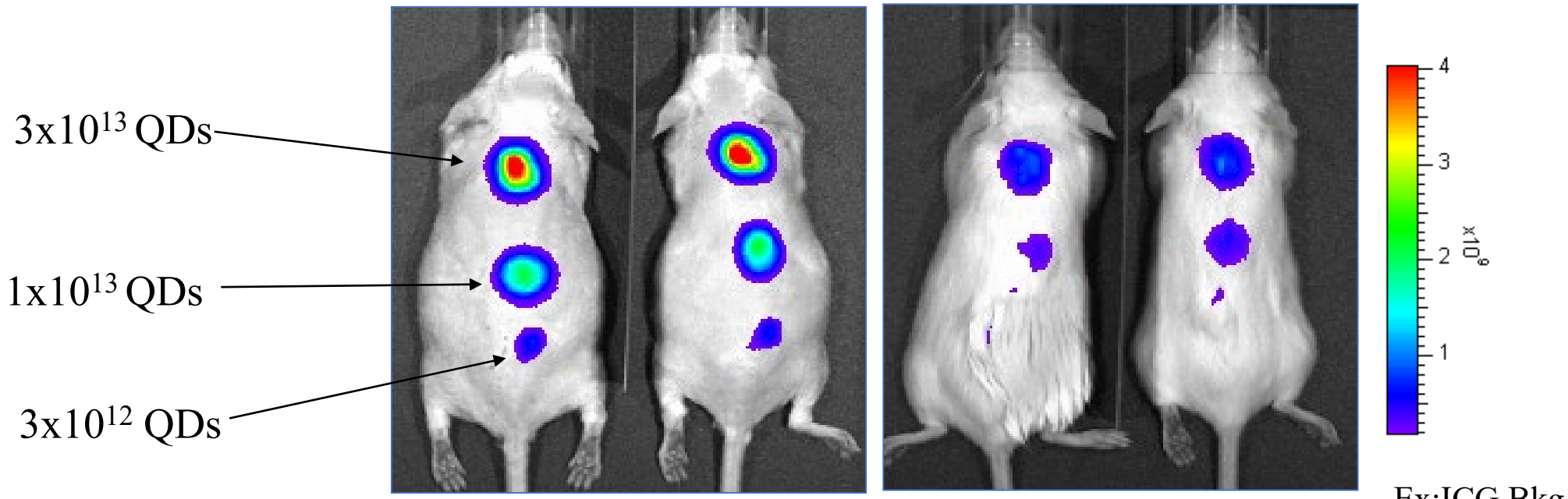
荧光成像小鼠剃毛——降低光吸收

QDs were injected subcutaneously in a 20 µl volume. Mice are FVB female on diet AIN-76A.



A four fold difference in signal!

使用近红外荧光探针也需要剃毛!

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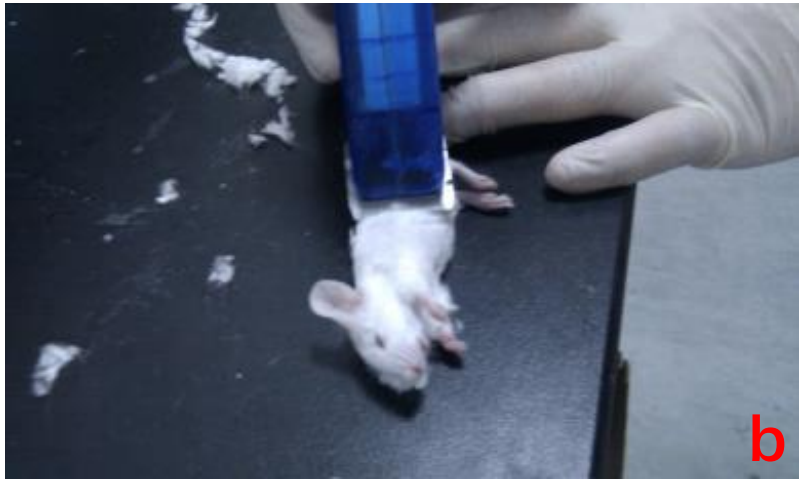
荧光成像小鼠剃毛——降低光吸收



(a) 不能用硫化钠溶液脱毛；
(b) 使用PerkinElmer剃毛器对小鼠进行剃毛；
(c) 使用剃毛器后的效果，基于剃毛刀头的设计，有约1 mm的体表绒毛无法剃除；
(d) 使用脱毛膏，将剩下的体表绒毛去除，用完脱毛膏后用纱布蘸温水檫拭小鼠，避免脱毛膏的自发荧光干扰。

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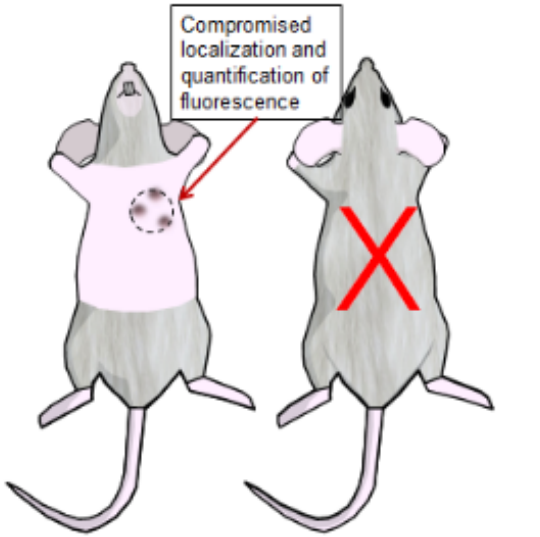
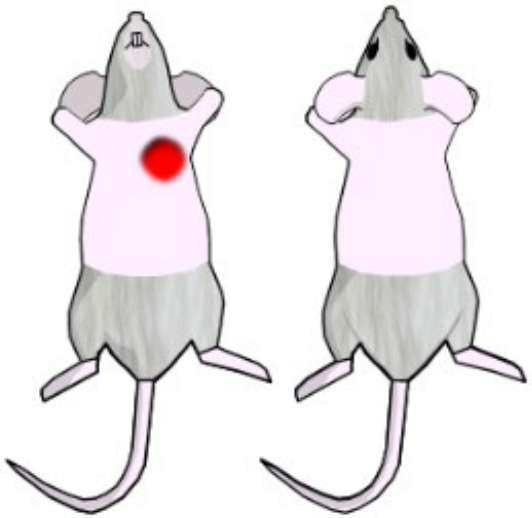
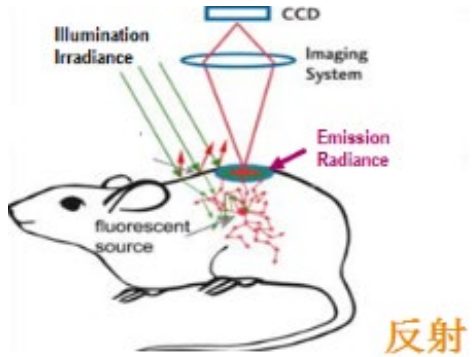
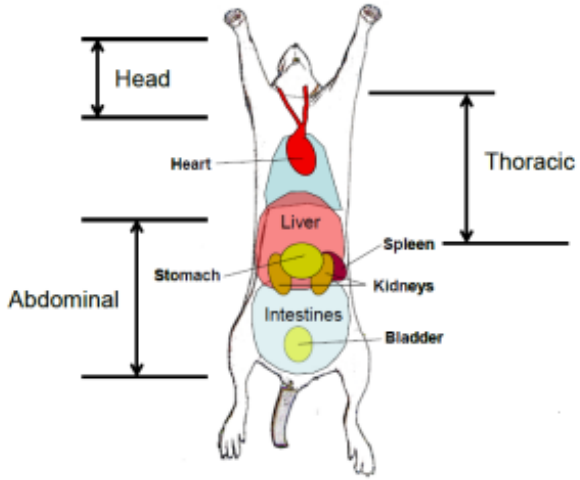
荧光成像小鼠剃毛——降低光吸收







- 荧光反射成像时一般只需剔除成像面侧毛



- 荧光透射成像时需剔除成像面两侧毛

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活体光学成像——应用概述

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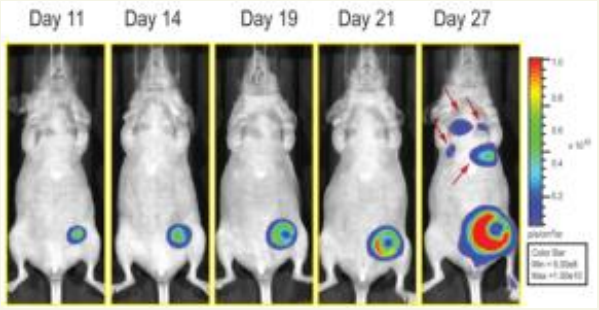
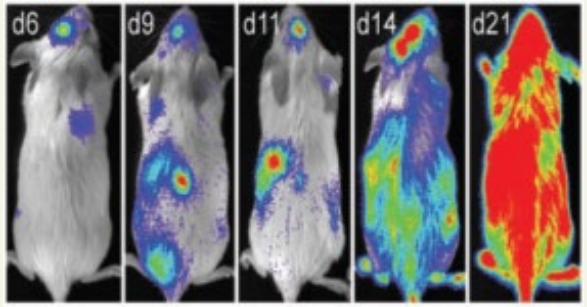
监测疾病的发生发展及治疗



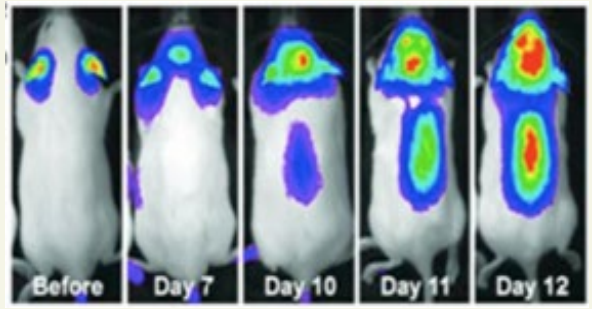
- 癌症
- 免疫性疾病
- 感染性疾病
- 代谢性疾病
- 心血管疾病
- 神经疾病

监测细胞动态变化



- 干细胞
- 免疫细胞
- 存活
- 分布
- 迁移
- 靶向

探究疾病分子机制



- 基因与疾病关系
- 疾病信号通路
- 细胞因子作用

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肿瘤相关应用

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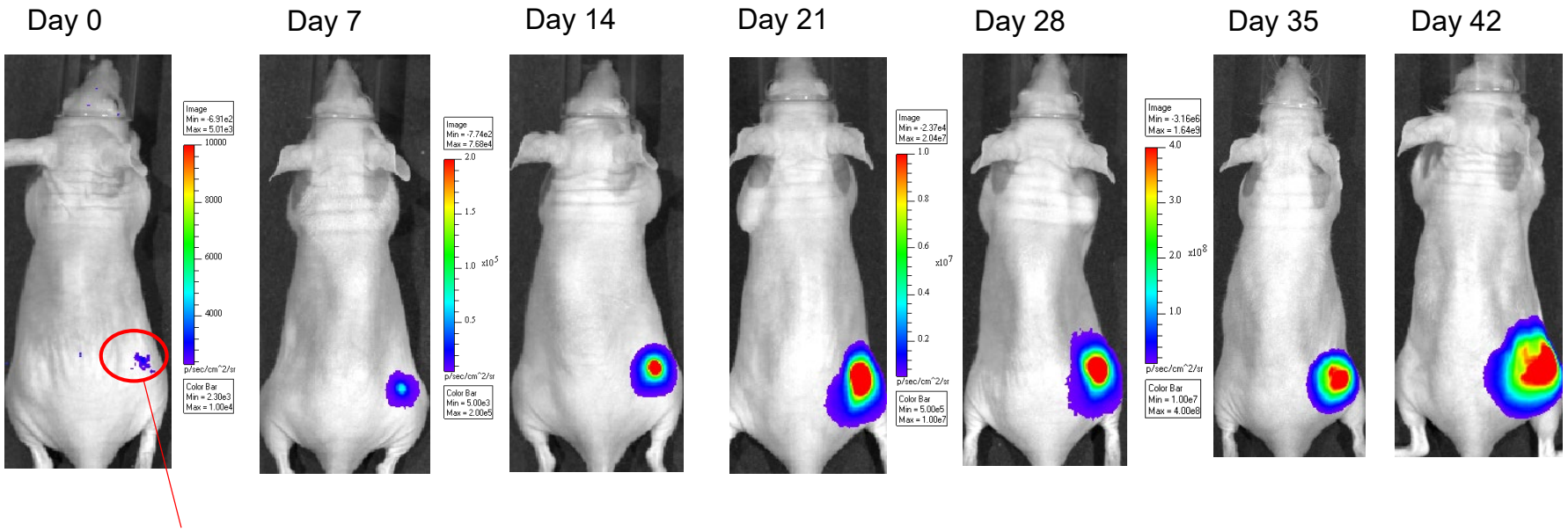
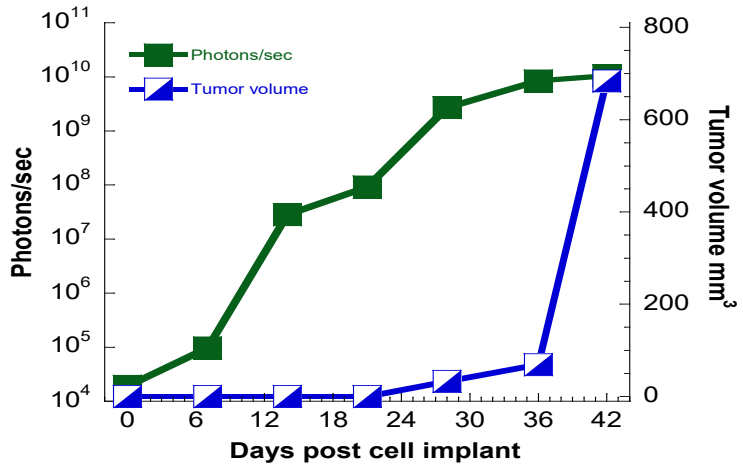
合理评价肿瘤的生长状态——“看的早”



高灵敏度：可监测微量肿瘤细胞和转移位点

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

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肿瘤相关应用

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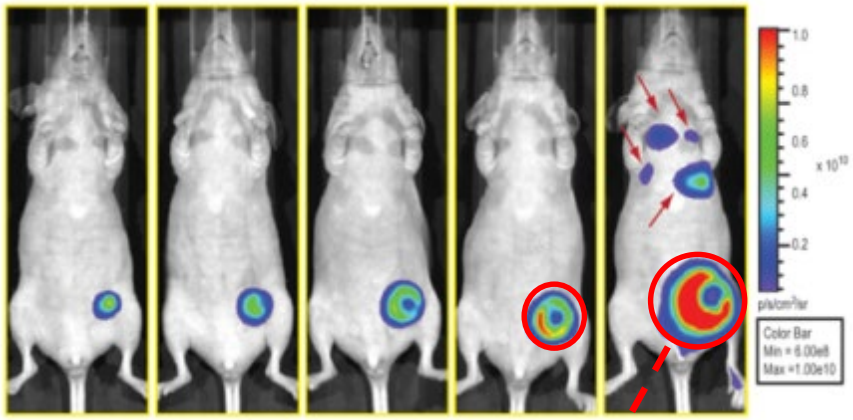
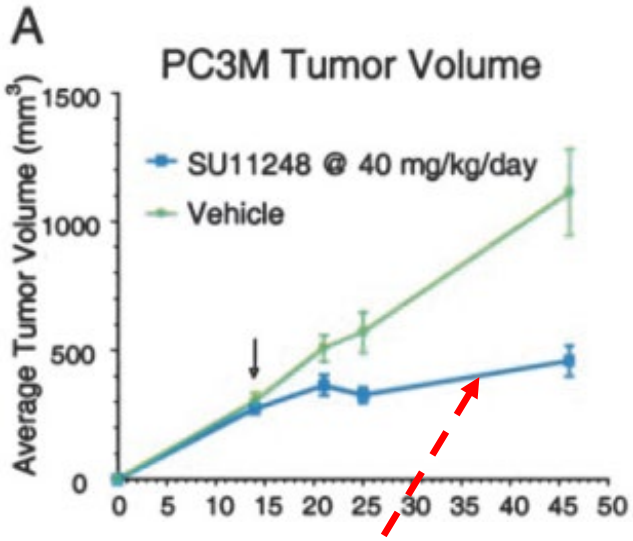
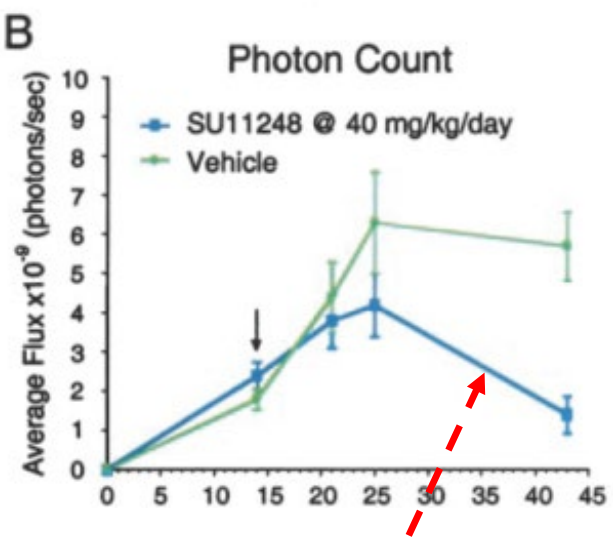
合理评价肿瘤的生长状态——“看的准”



瘤体虽然在变大，但实际内部已经凋亡坏死



Physical measurement
(tumor still getting bigger)



Bio-photonic imaging
(tumor cells being killed)

Mendel *et al* 2003

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抗肿瘤药物评价

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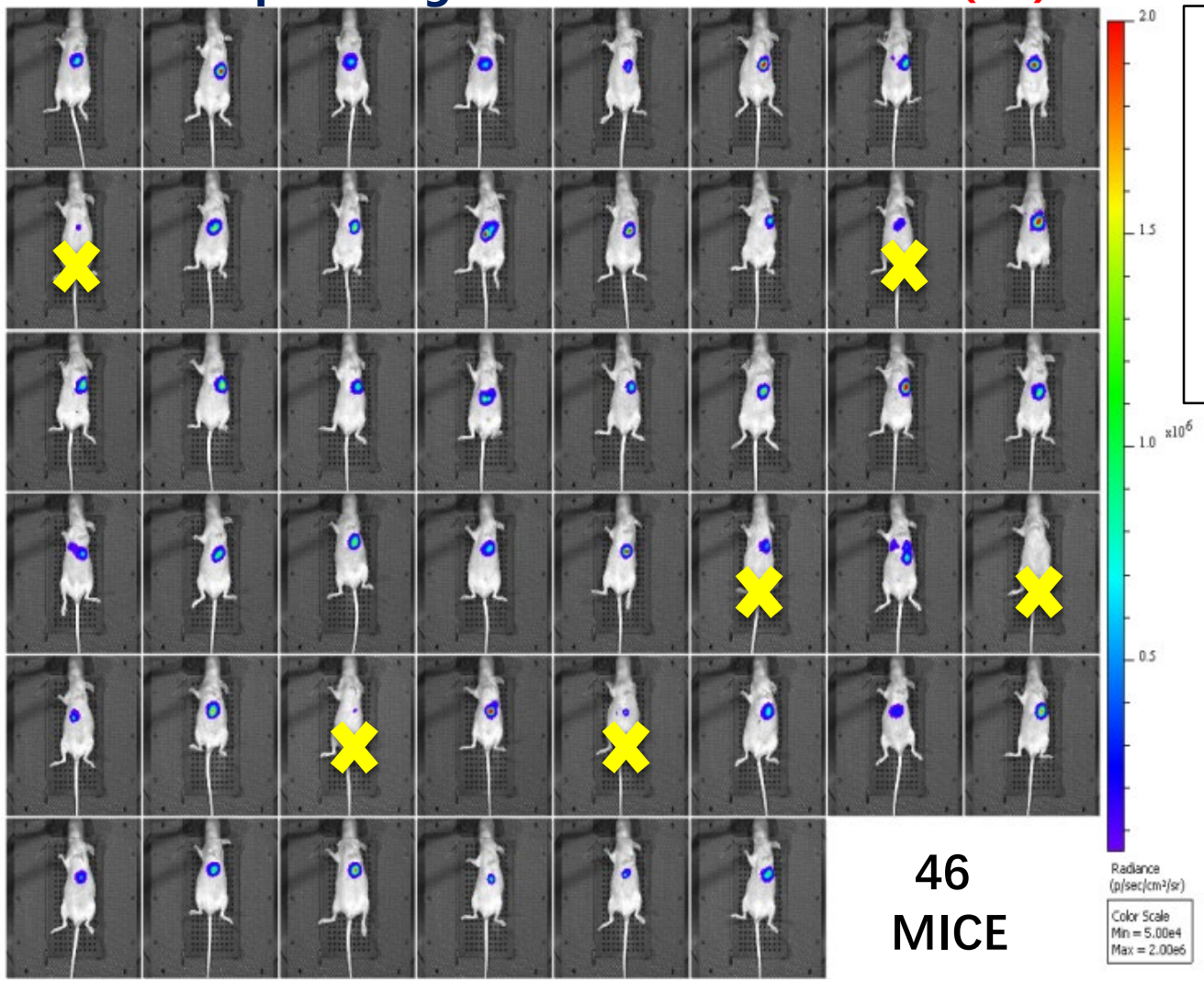
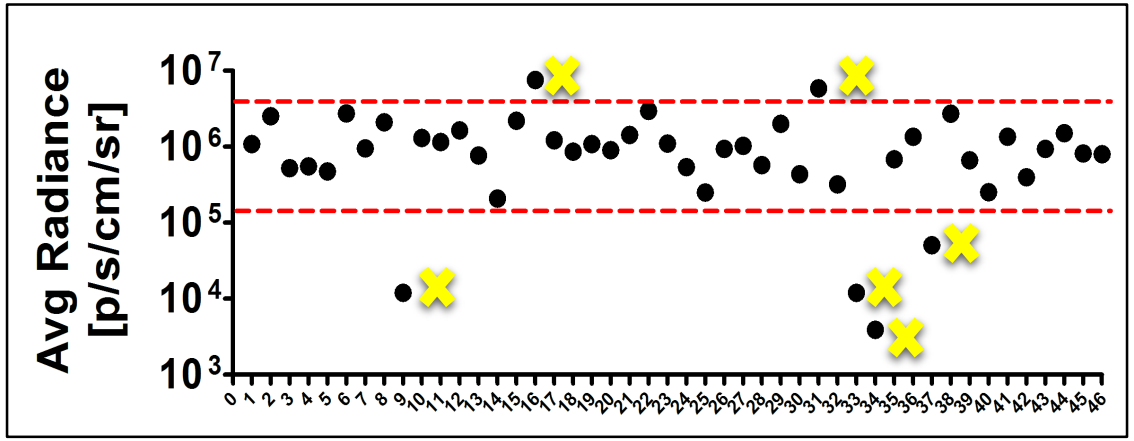
为实验分组提供合理依据

Orthotopic Xenograft Model of Liver Tumor (BLI)



46 MICE



对入组进行实验的造模动物进行肿瘤大小的筛查，剔除偏离均值过多的实验对象

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抗肿瘤药物评价

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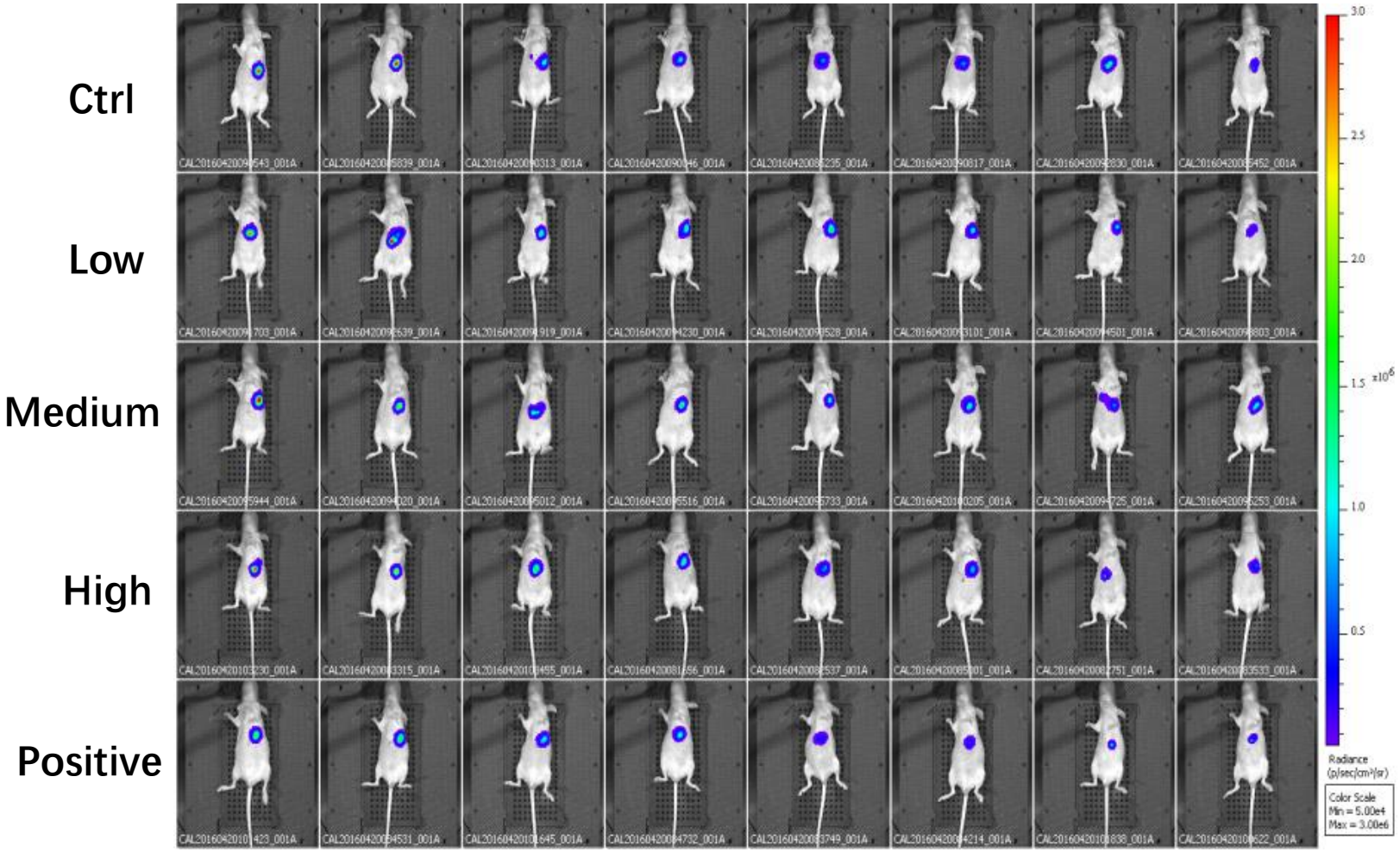
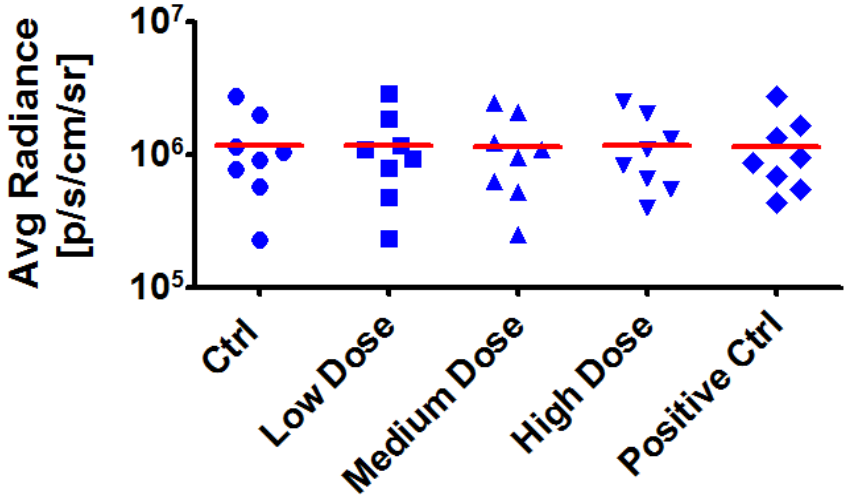
Randomized Grouping





40 MICE

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抗肿瘤药物评价

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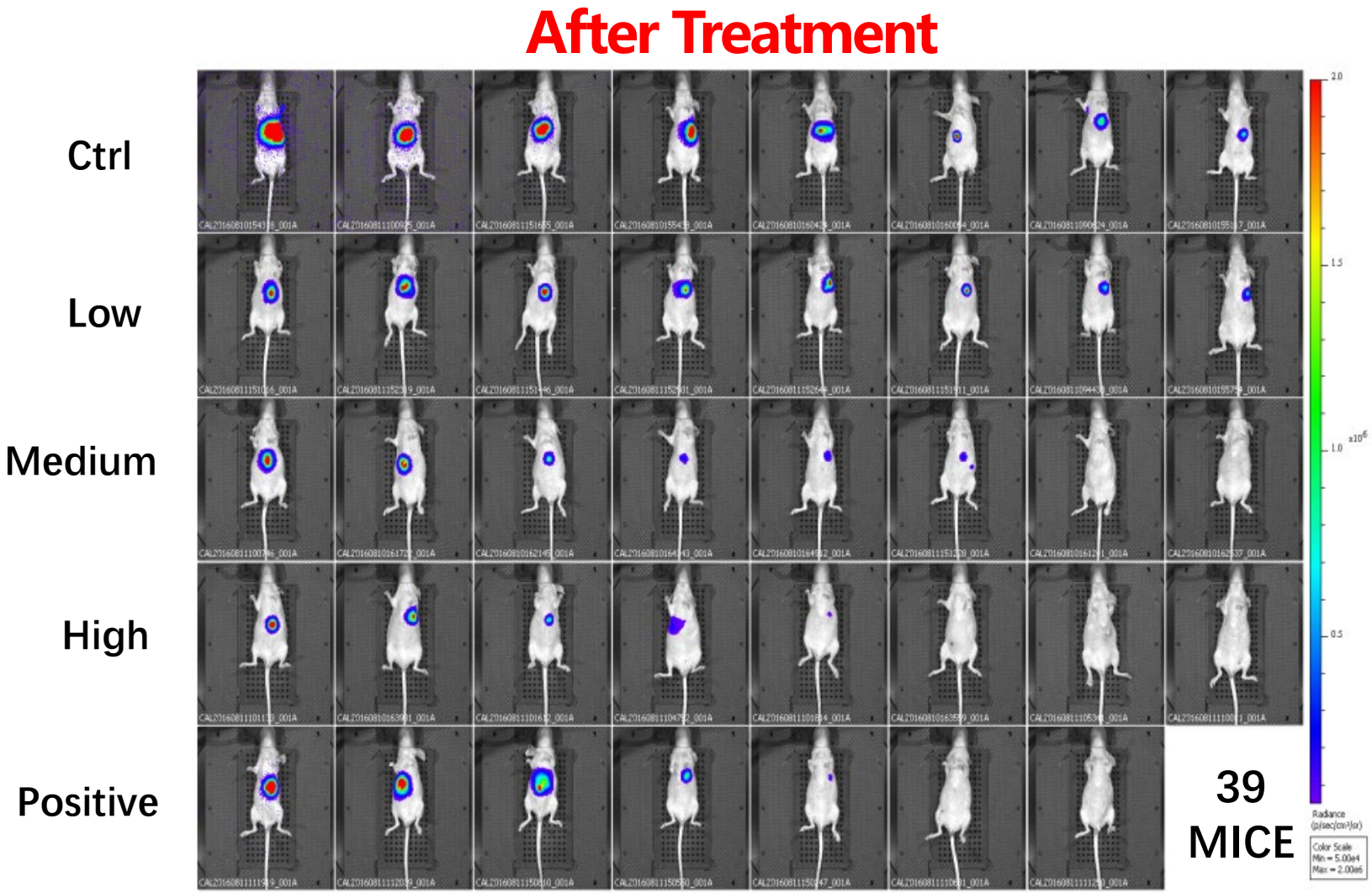
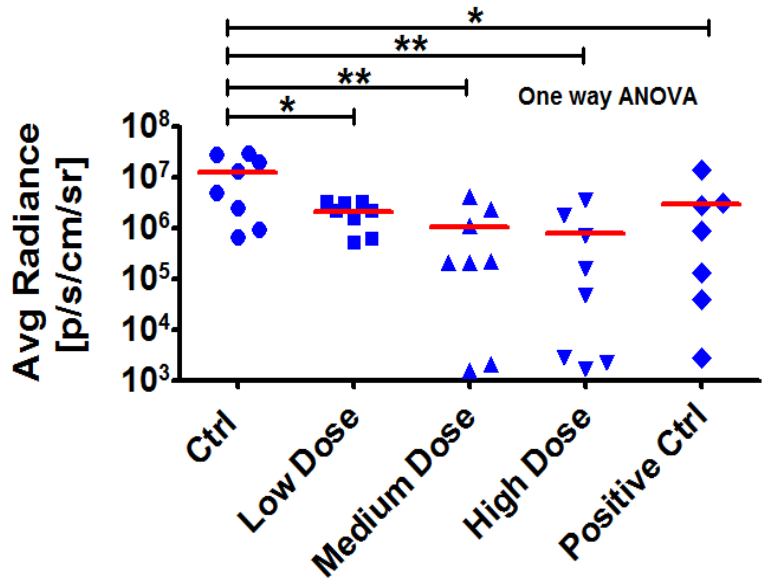
为实验分组提供合理依据





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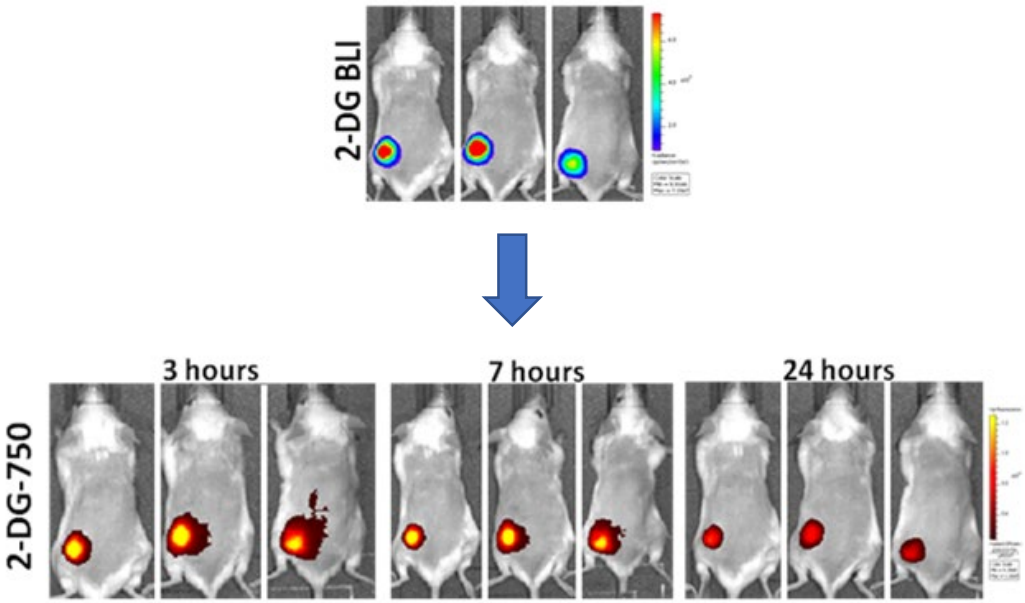
荧光探针指示肿瘤等疾病发生

	血管生成	细胞凋亡	关节炎	动脉粥样硬化	骨代谢	心血管疾病	高血压	感染疾病	急性炎症	肾功能检测	肿瘤	肺部疾病	血管疾病
利用酶激活类荧光试剂探究疾病发展过程中的相关分子生物学事件													
Cat B 680 and 750 FAST™			•	•		•			•		•	•	•
Cat K 680 FAST			•		•						•	•	
MMPSense® 680, 750 FAST, and 645 FAST			•	•		•			•		•	•	•
利用靶向类荧光试剂实现对研究对象的靶定、观测及分析													
2-DG 750 probe											•		
Annexin-Vivo 750		•		•							•		
BacteriSense™ 645								•					
Bacterial Detection Probe 750								•					
COX-2 probe			•						•		•		
FolateRSense™ 680			•						•		•		
IntegriSense™ 680, 750 and 645	•			•		•			•		•		•
HER2Sense™ 645											•		
利用血管生理类荧光试剂观测肿瘤及炎症病灶点的血管新生或渗漏及血池形成													
AngioSense® 680 EX and 750 EX	•		•			•			•		•	•	•
AngioSPARK® 680 and 750			•	•		•	•		•		•		•
Superhance™ 680	•		•			•			•		•		•

Tumor targeting with both Biolumenescence and RediJect 2-DG 750



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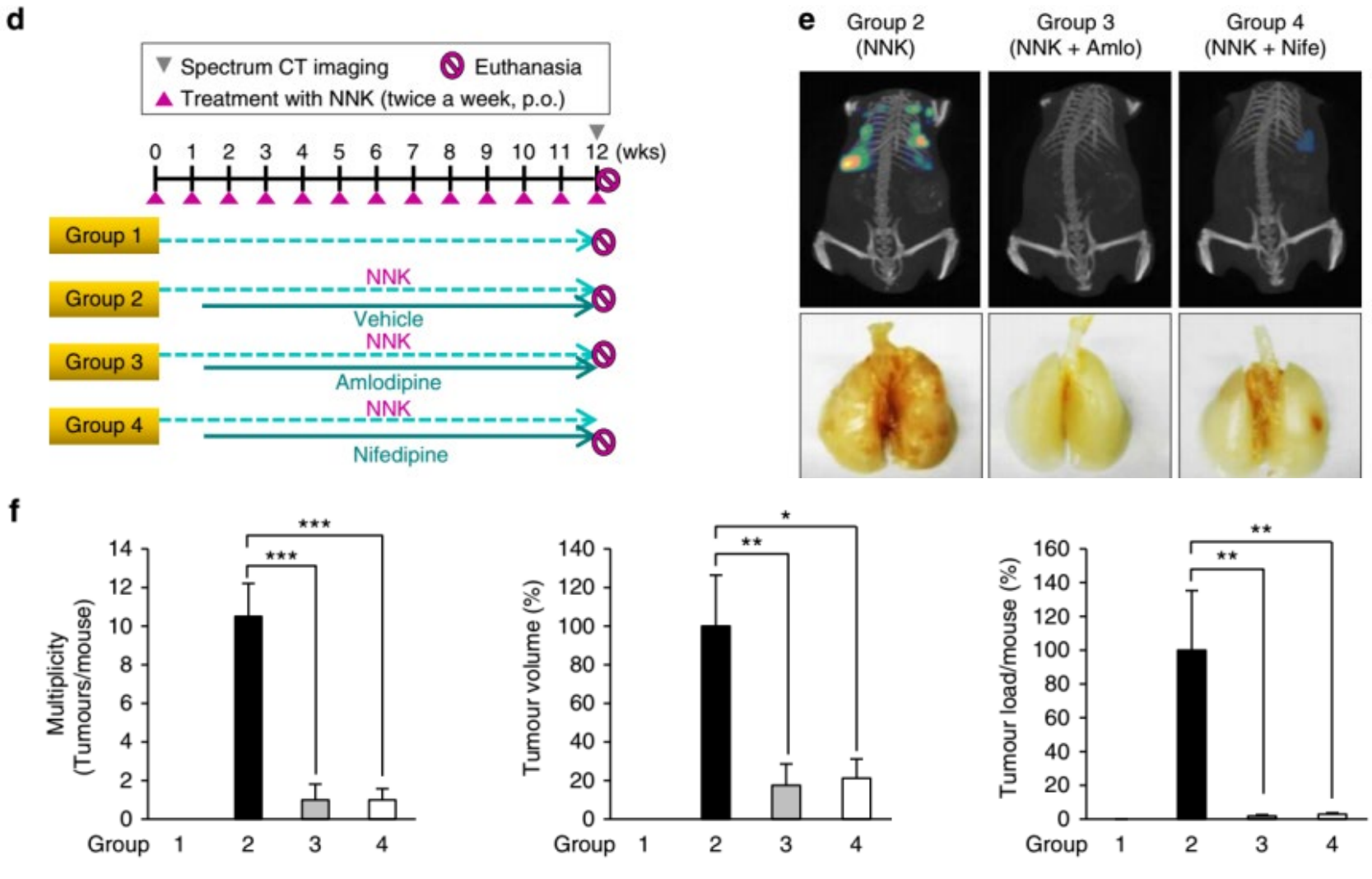
抗肿瘤药物评价



MMPSense 680 probe

Amlodipine: 氯胺地平
Nifedipine: 硝苯地平

***Hye-Jin Boo. et al.*, NATURE COMMUNICATIONS.2016**

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药物靶向分布

Specific targeting effects of ICG-Cetuximab

ICG-Cetuxi

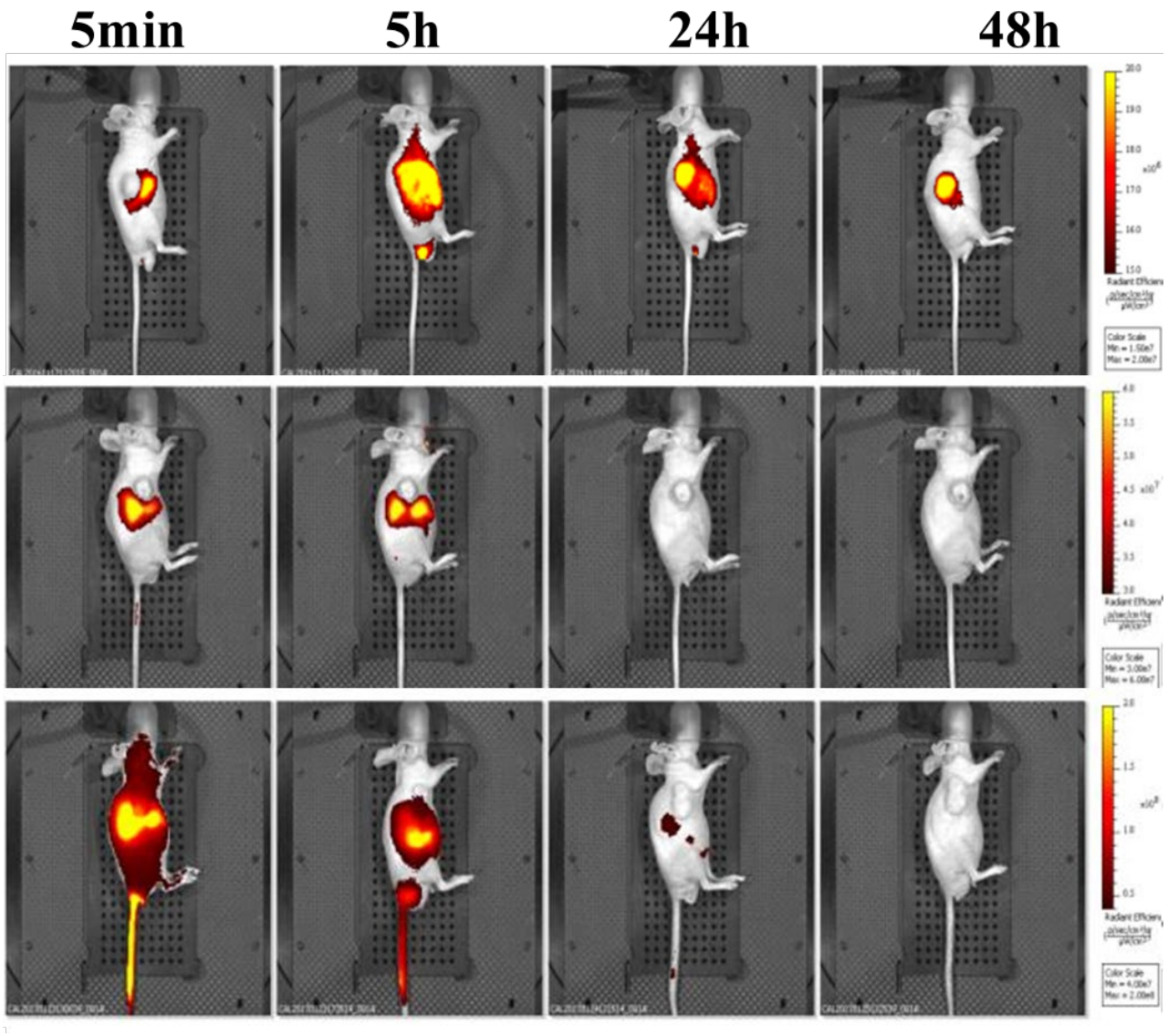
Lovo EGFR(+)

ICG-IgG

Lovo EGFR(+)

ICG-Cetuxi

Sw620 EGFR(-)



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药物靶向分布

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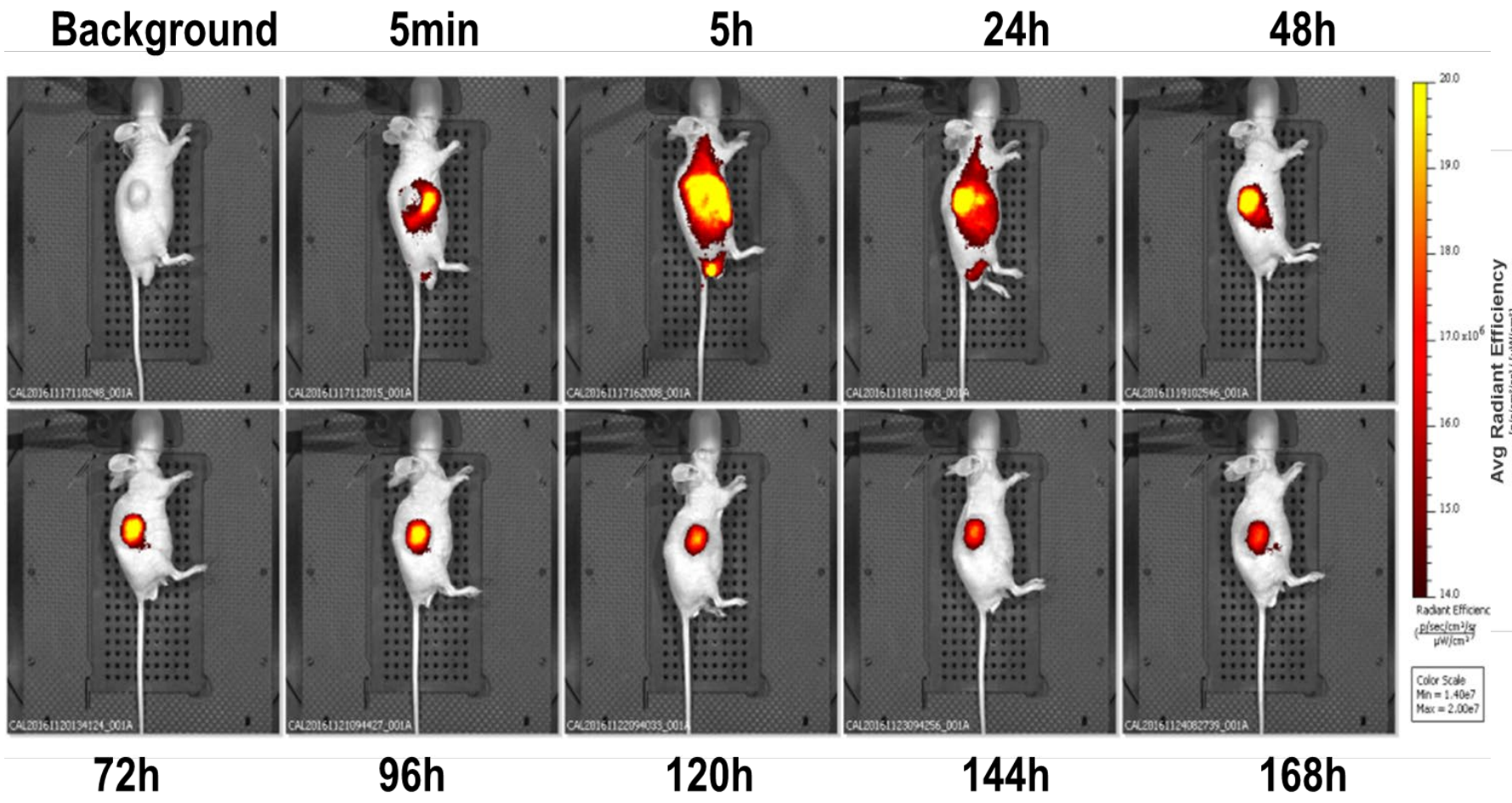
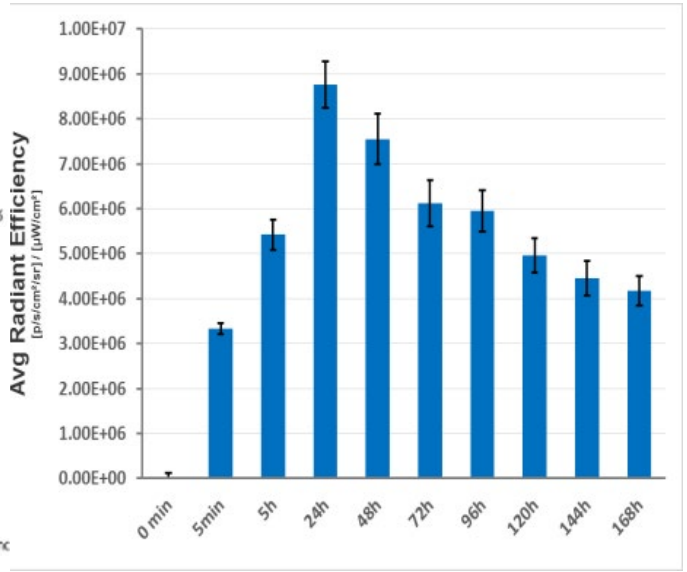
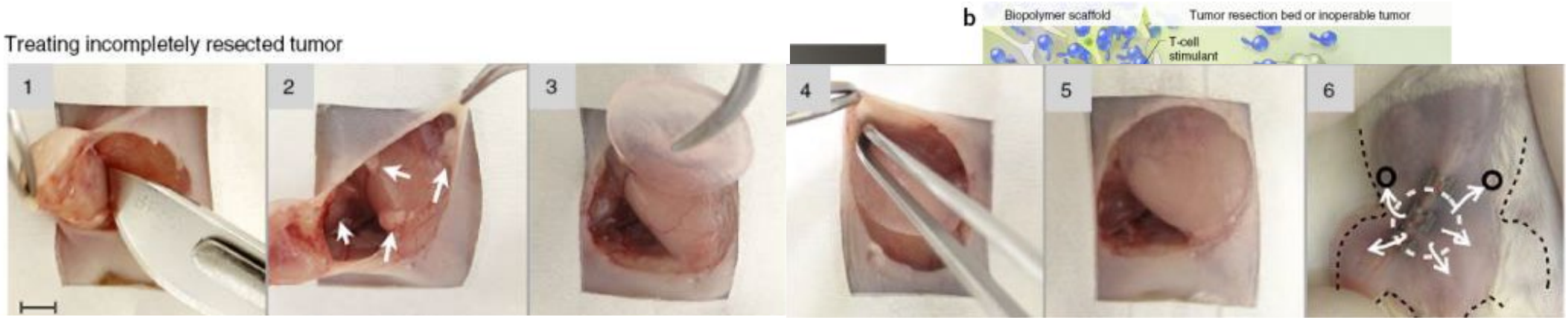
Prolonged targeting effects of ICG-Cetuximab

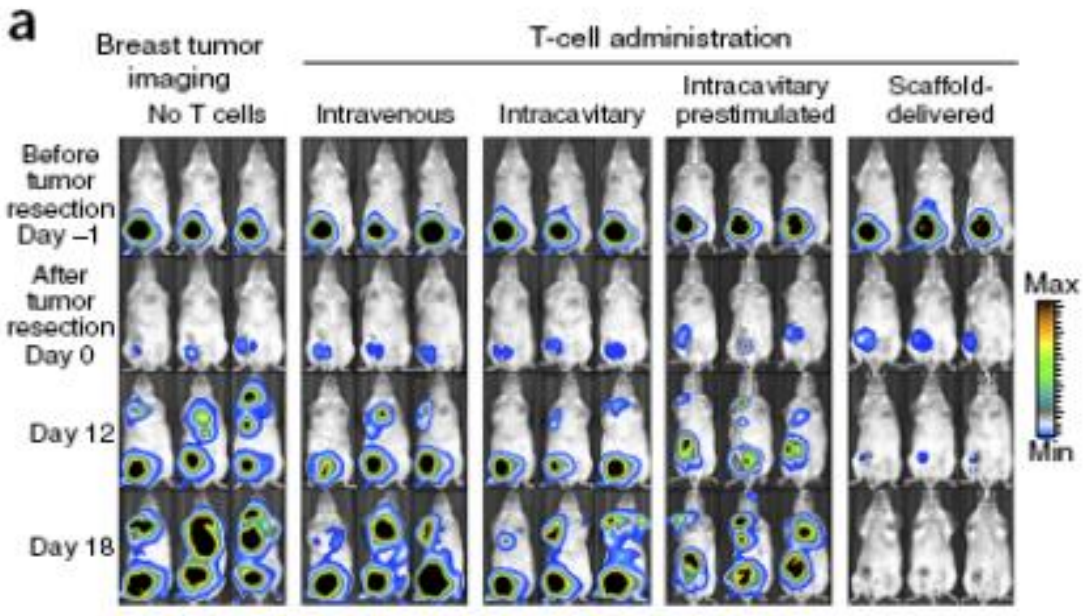


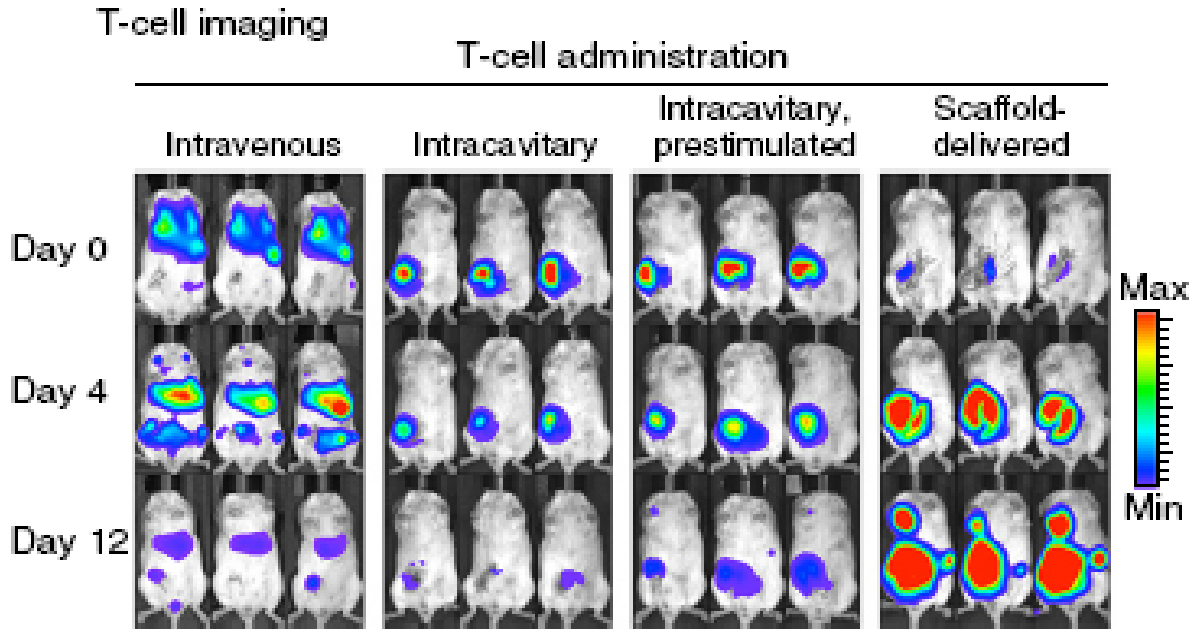


Xing.Huang@perkinelmer.com

细胞存活、靶向与细胞治疗







Sirkka B Stephan, et al, Nature biotechnology,2015.

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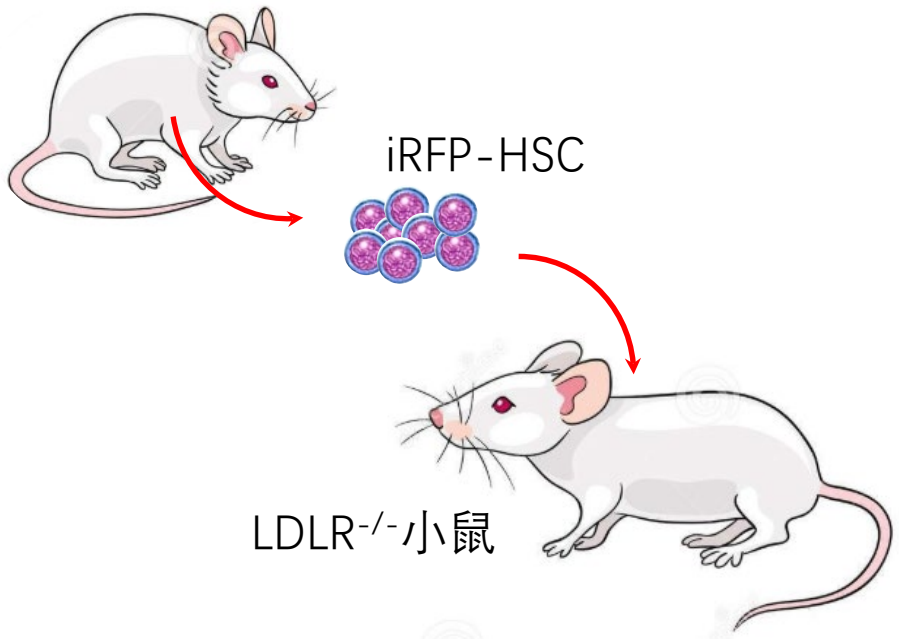
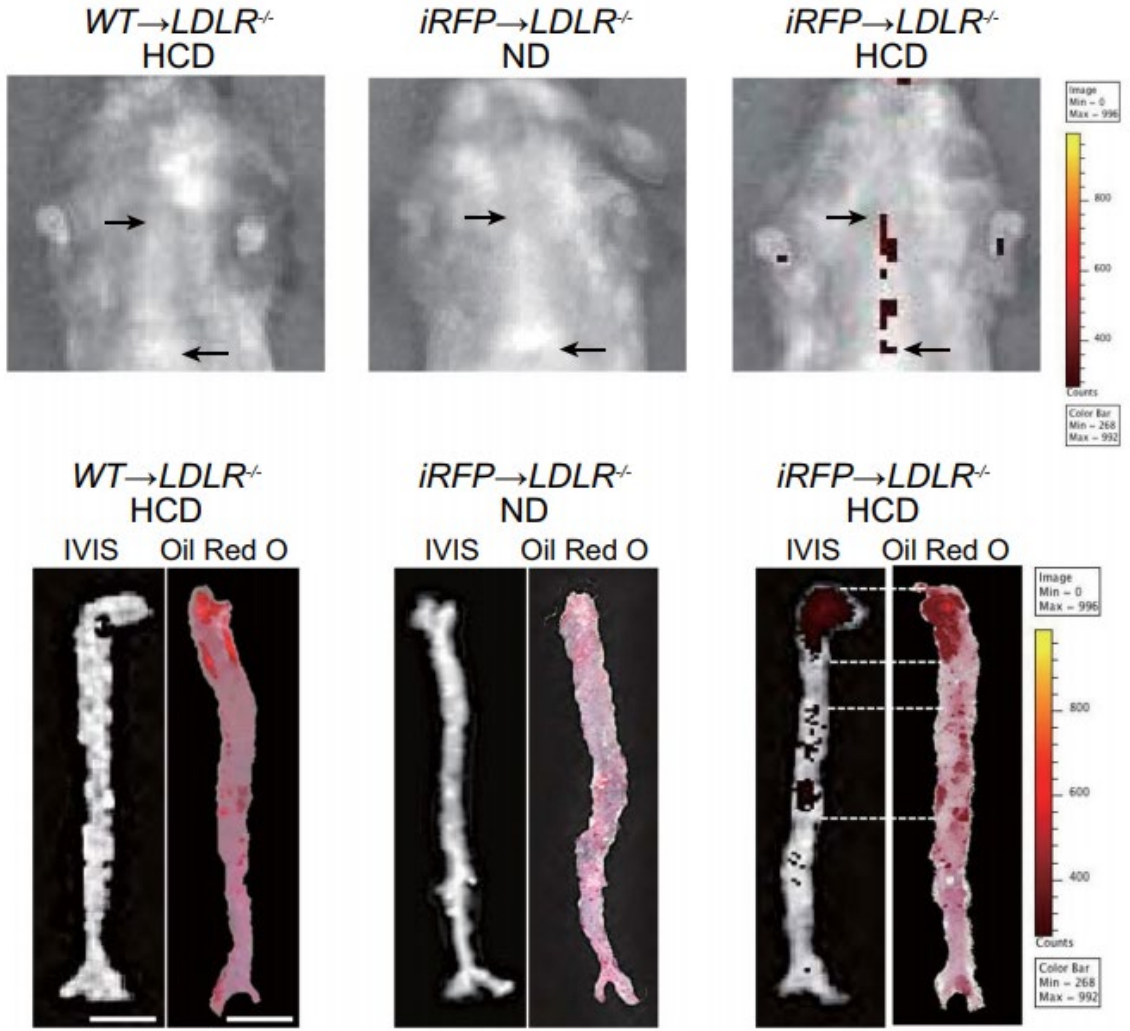
荧光细胞示踪动脉粥样硬化

全身表达iRFP小鼠



LDLR: 低密度脂蛋白受体

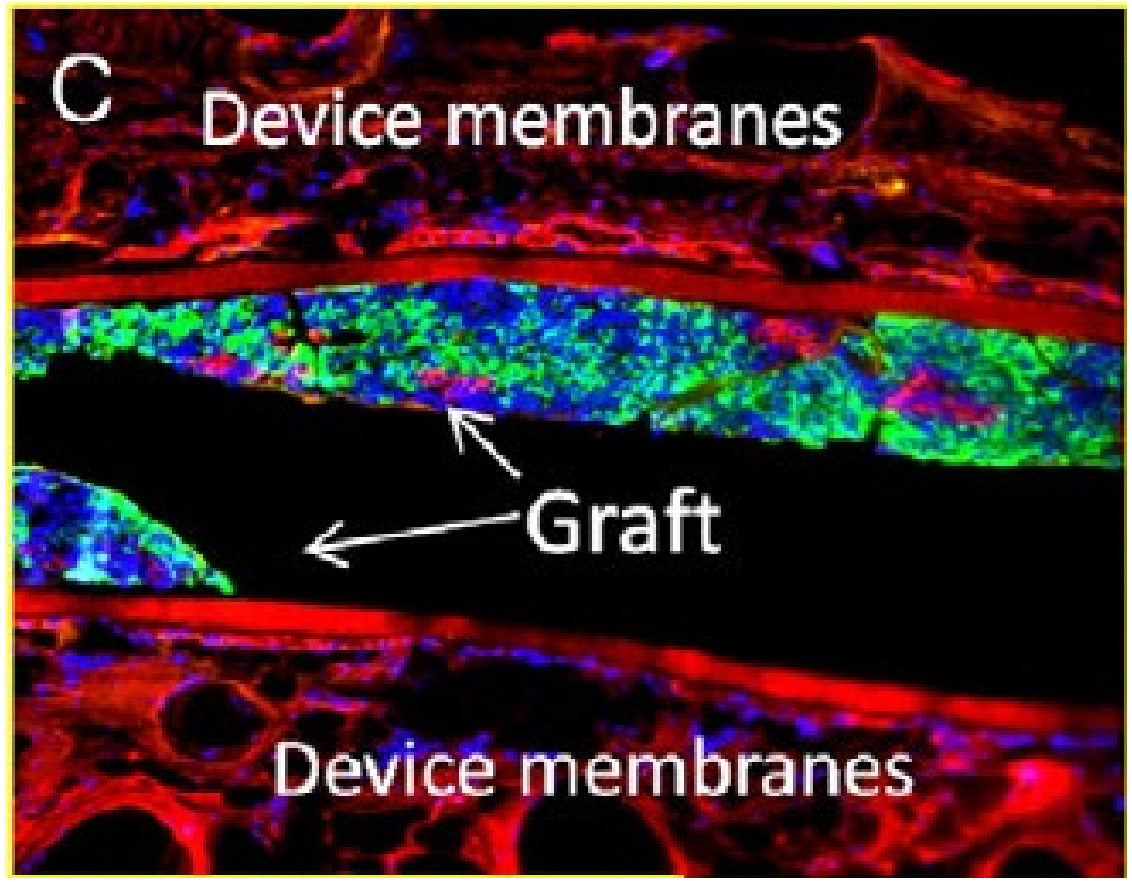
HCD: 高胆固醇饮食



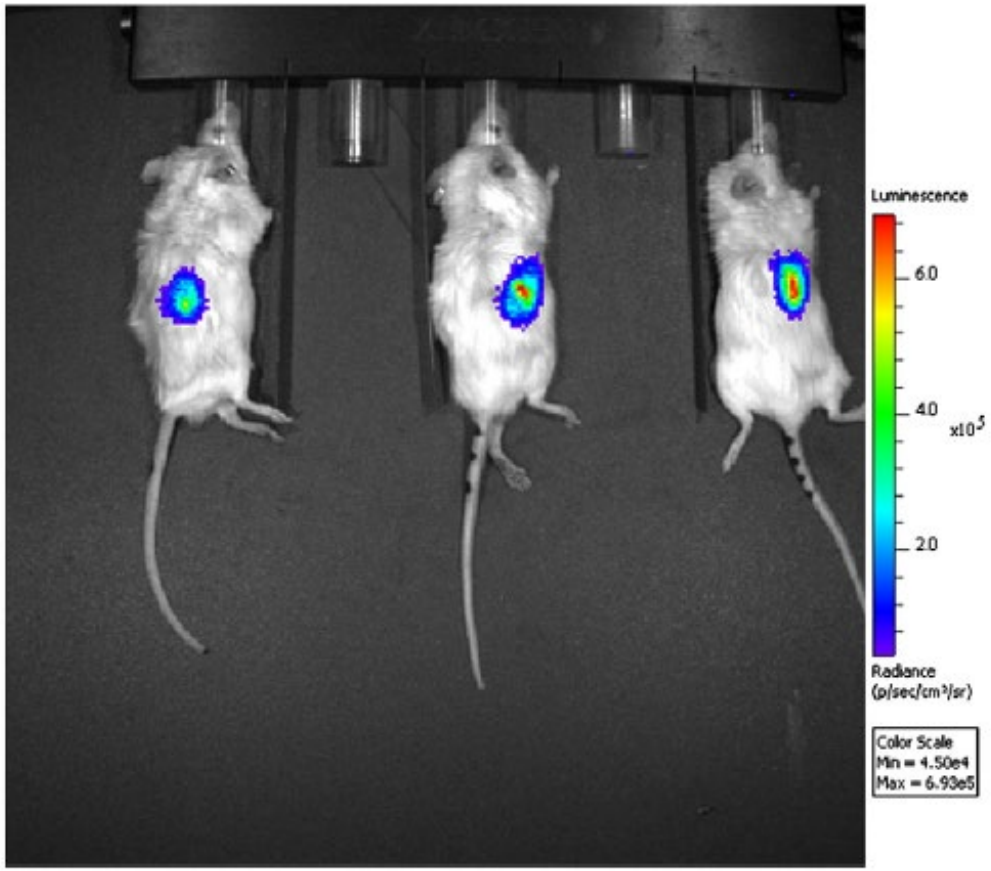
Kaushalya Kulathunga et al.,Scientific Reports 8: 14515 (2018)

Xing.Huang@perkinelmer.com

细胞存活、靶向与细胞治疗



聚四氟乙烯（PTFE）双层封装膜系统
内表达胰岛素的细胞



IVIS系统监测植入的封装膜系统
内胰岛细胞的活性及位置变化

Kirk *et al*, Stem Cell Research, 2014

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病原微生物感染应用

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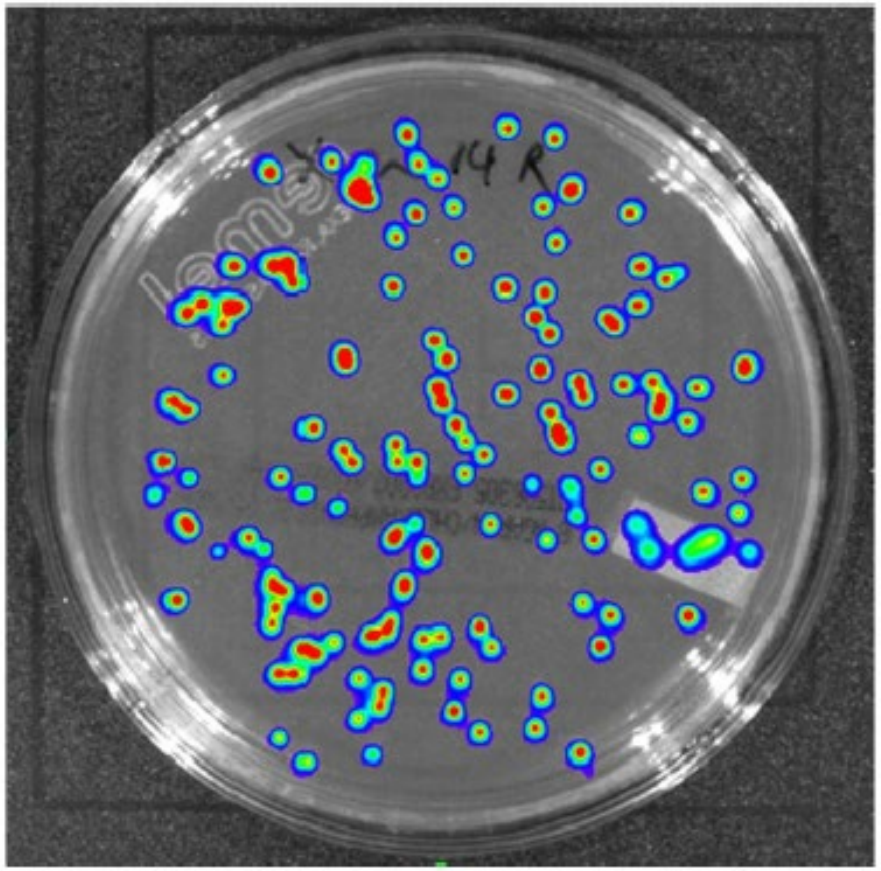
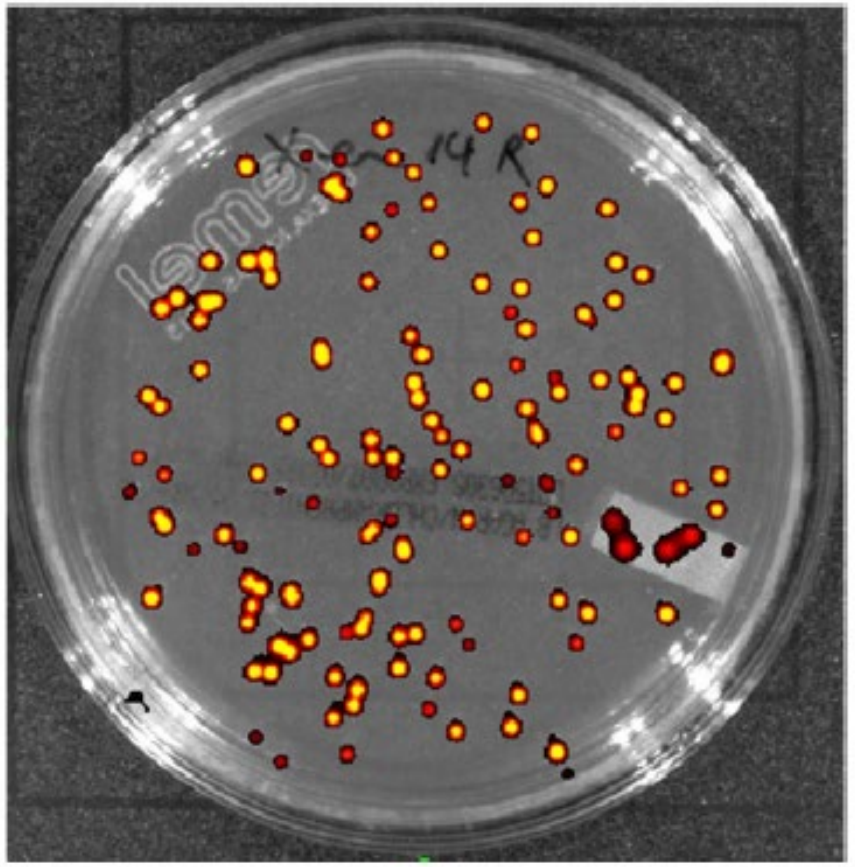
Bioluminescent Imaging



Fluorescent Imaging



利用细菌的基因表达能力进行标记，直接观察药物对细菌感染的治疗作用。

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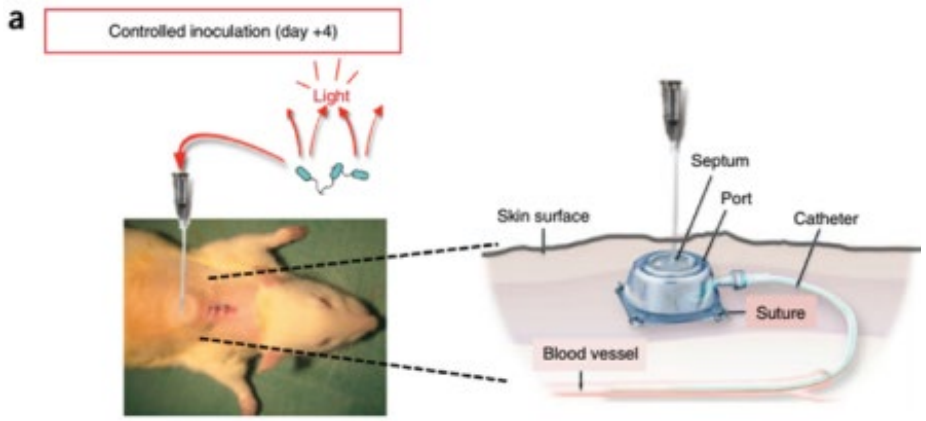
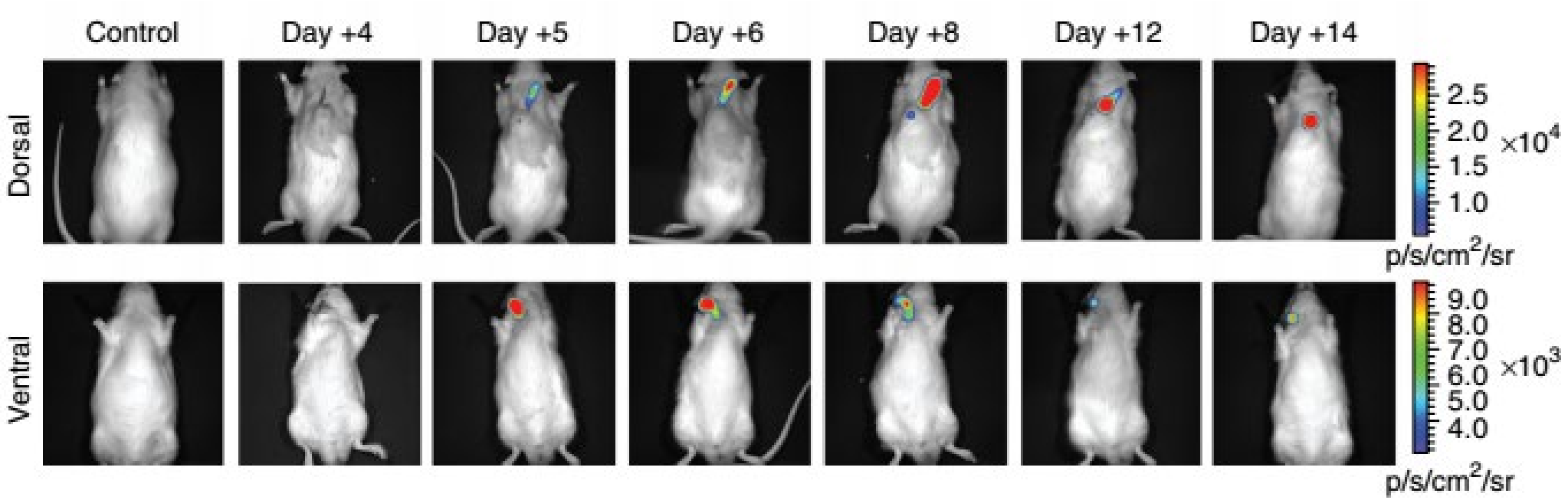
病原微生物感染应用



大鼠植入式静脉插管(TIVAPS)方式感染表皮葡萄球菌(S. epidermidis)，模拟临床上院内细菌感染过程，该模型可用于评价抗生素对感染的抑制效果，并已经用于头孢等抗生素效果评价。



Ashwini Chauhan， et al, **nature protocols., 2016**

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心血管疾病相关应用

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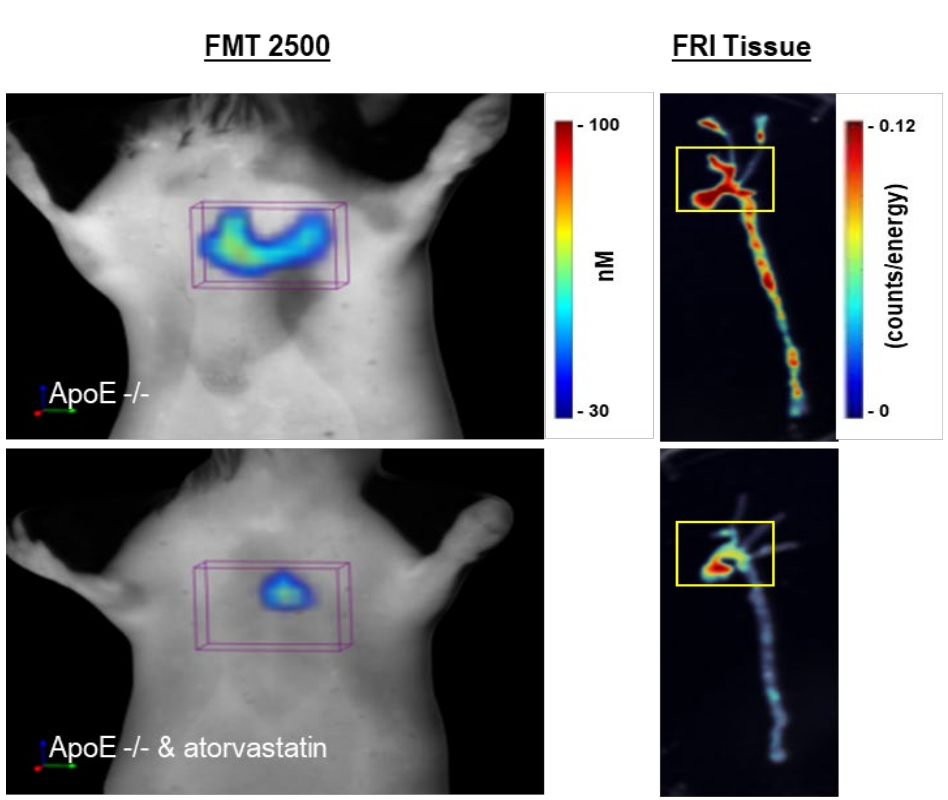
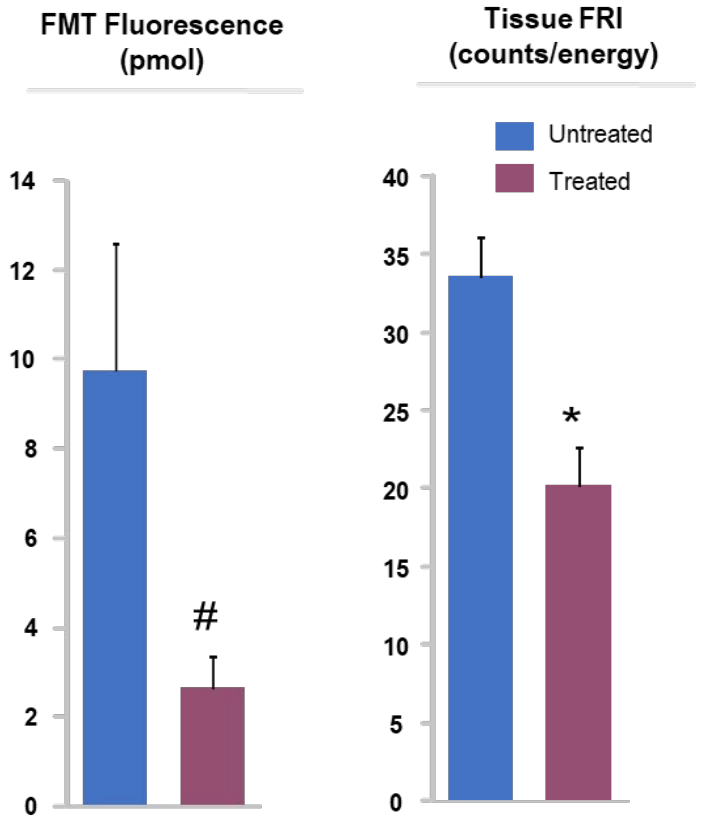
ProSense750: Prophylactic Atorvastatin Treatment



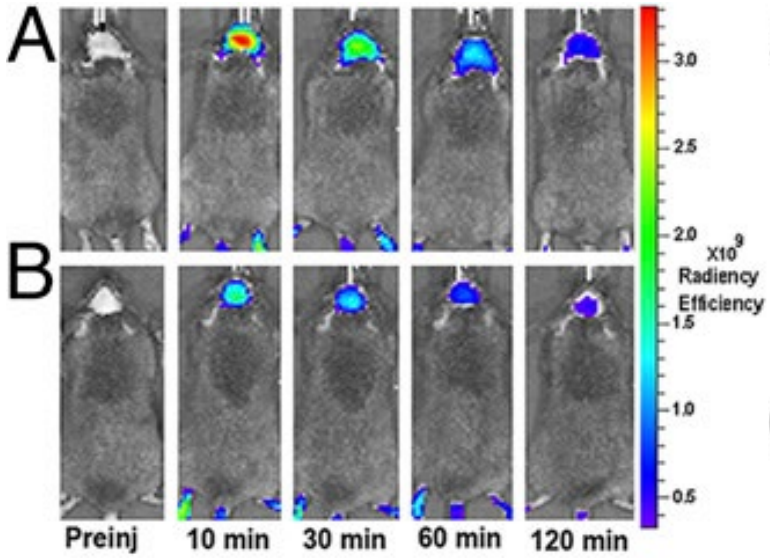


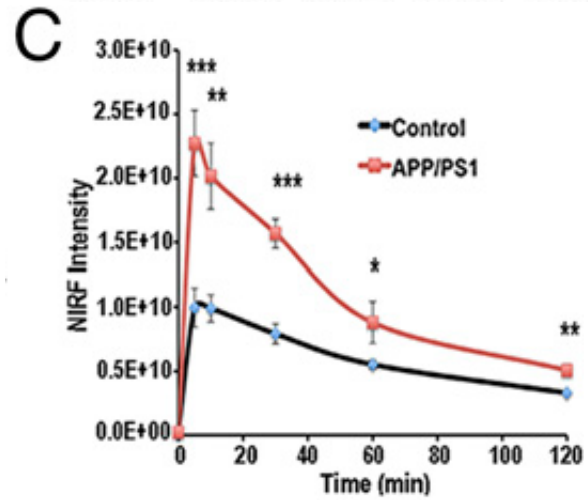
ApoE敲除的动脉粥样硬化模型小鼠在阿托伐他汀治疗后的恢复效果

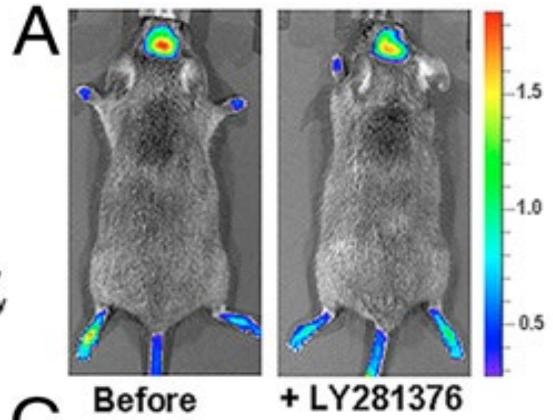
ProSense750 quantifies differences in signal associated with treatment efficacy

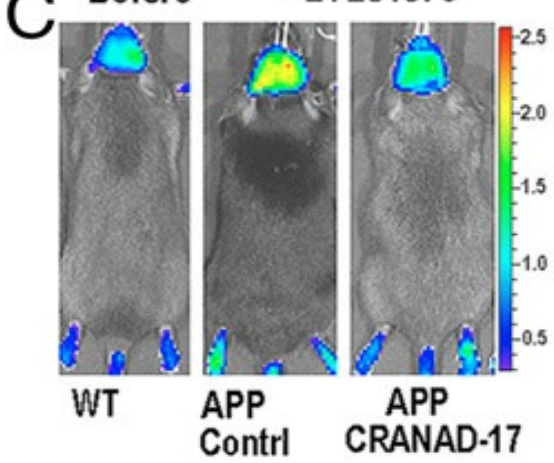
Xing.Huang@perkinelmer.com

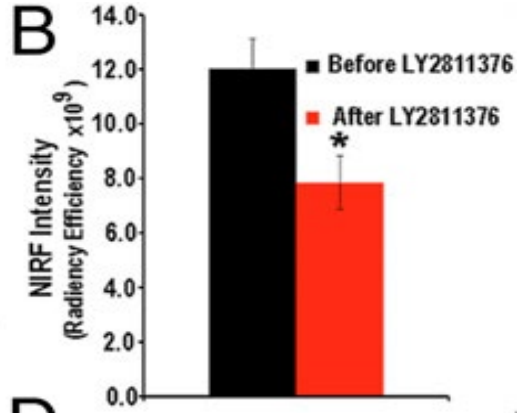
神经系统疾病相关应用

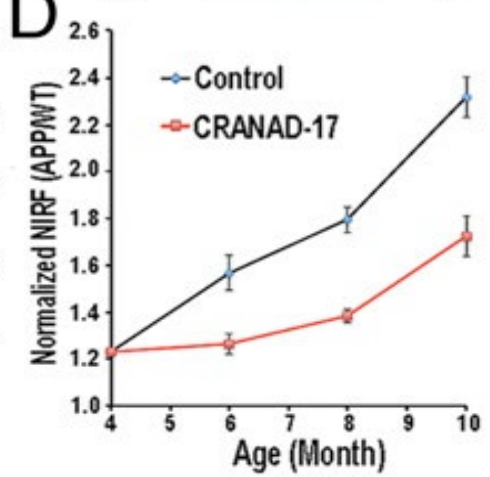












Alzheimer's disease

用IVIS活体成像系统评价药物治疗AD的效果

Zhang *et al*, PNAS, 2015

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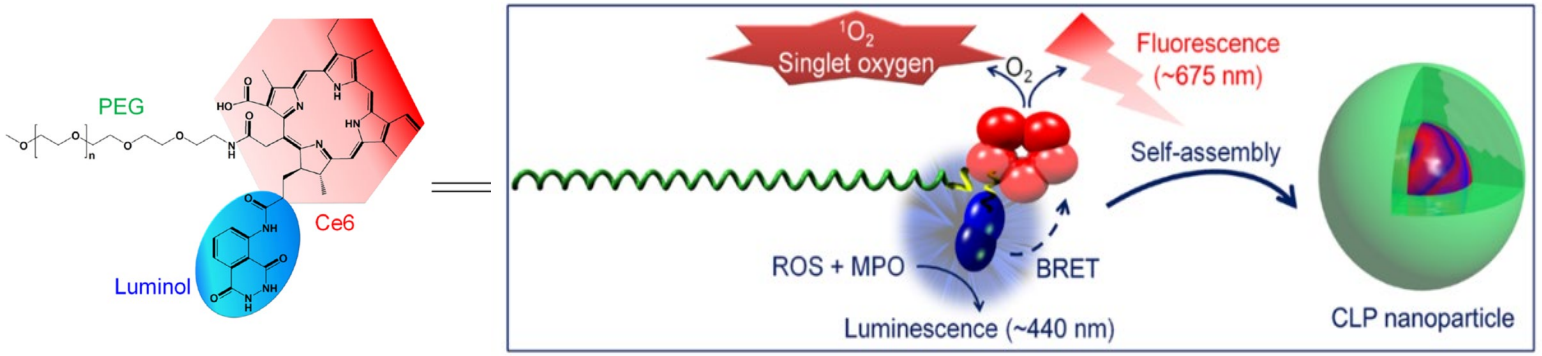
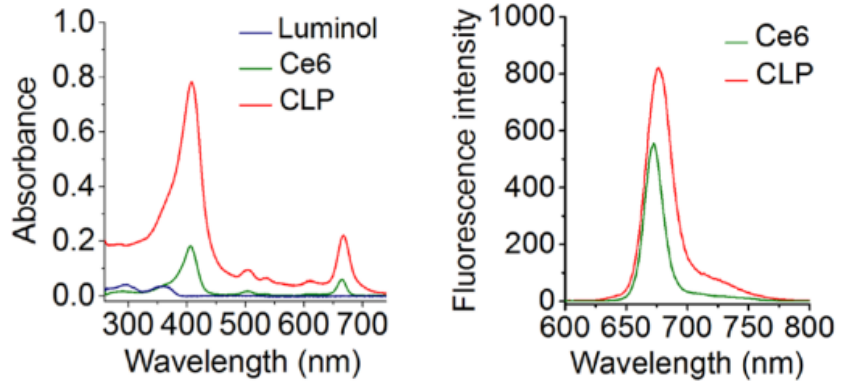
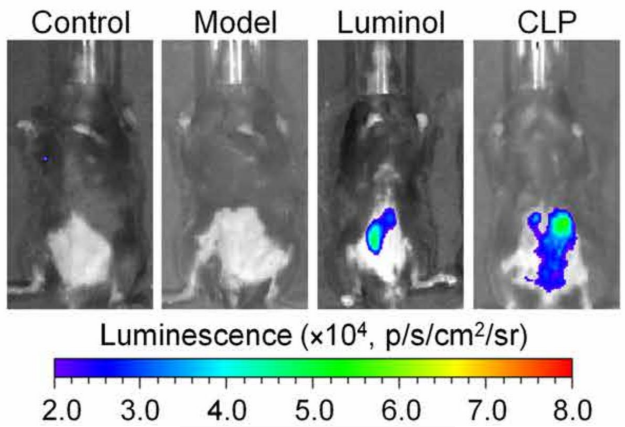
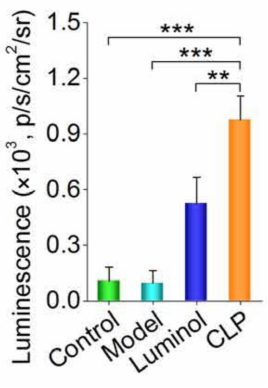
活性氧检测——成像与光动力治疗双功能探针设计

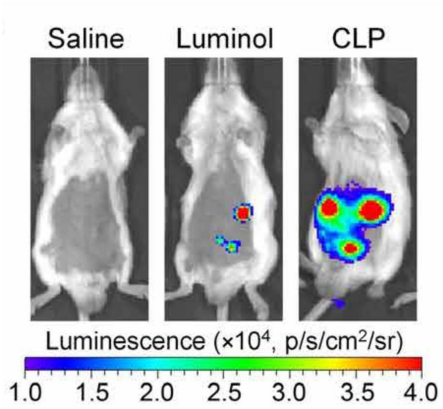




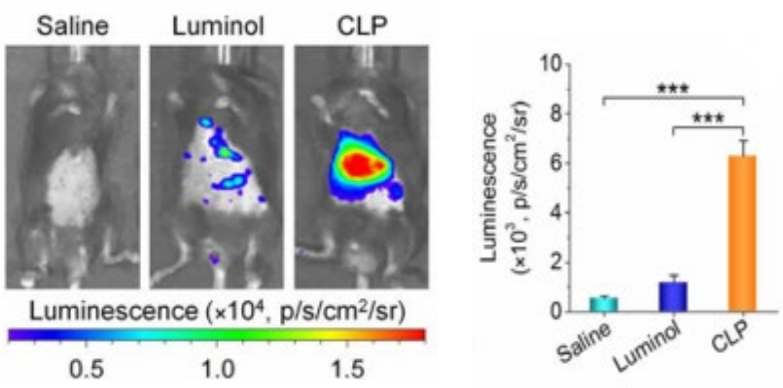


结肠炎模型





腹膜炎模型



急性肝损伤模型

Science Advances 2019; 5：2953

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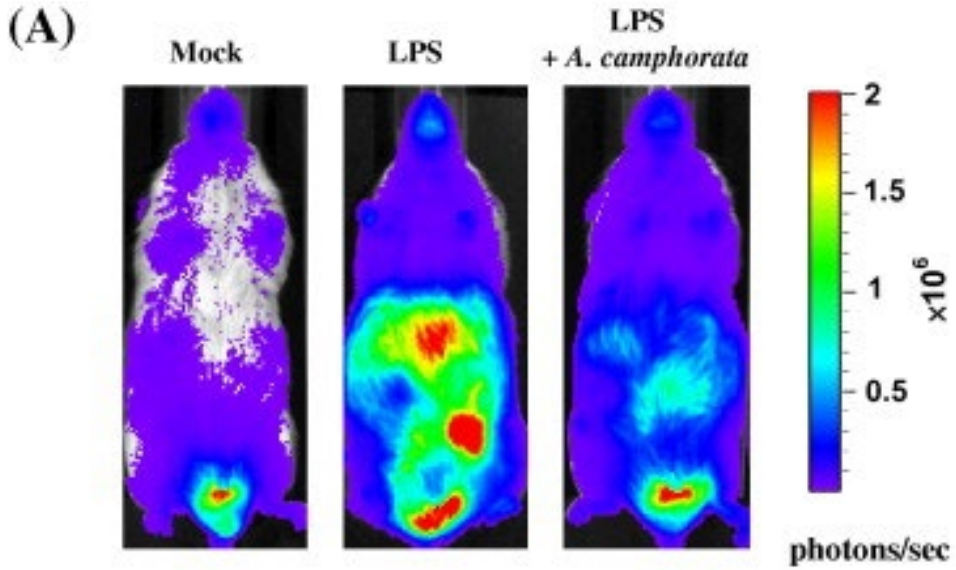
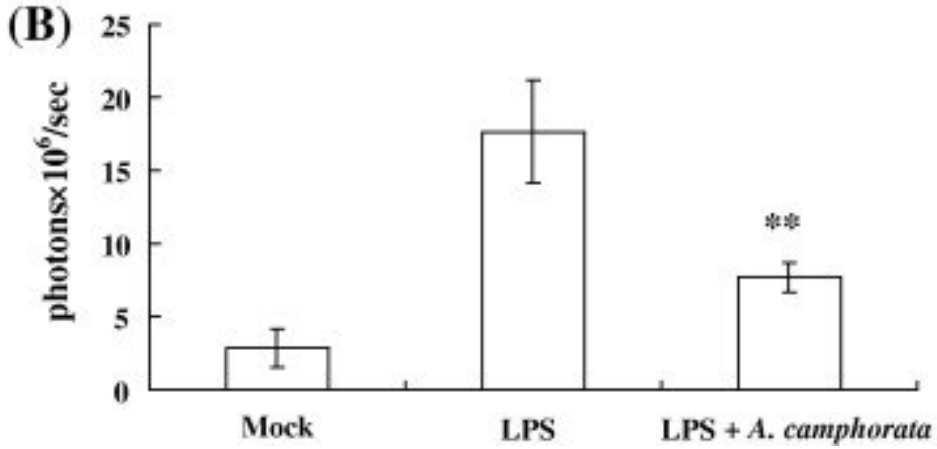
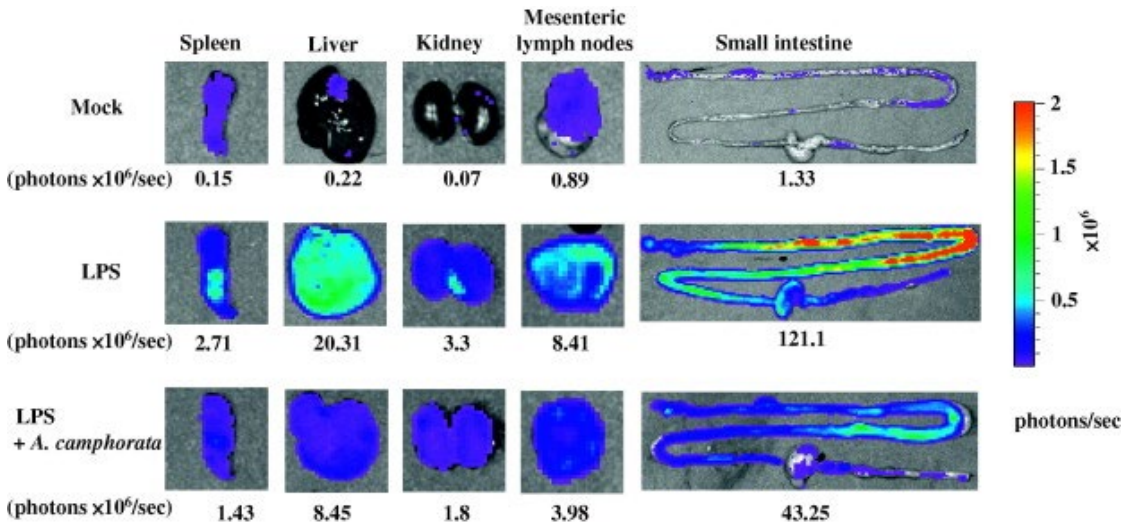
炎症相关应用





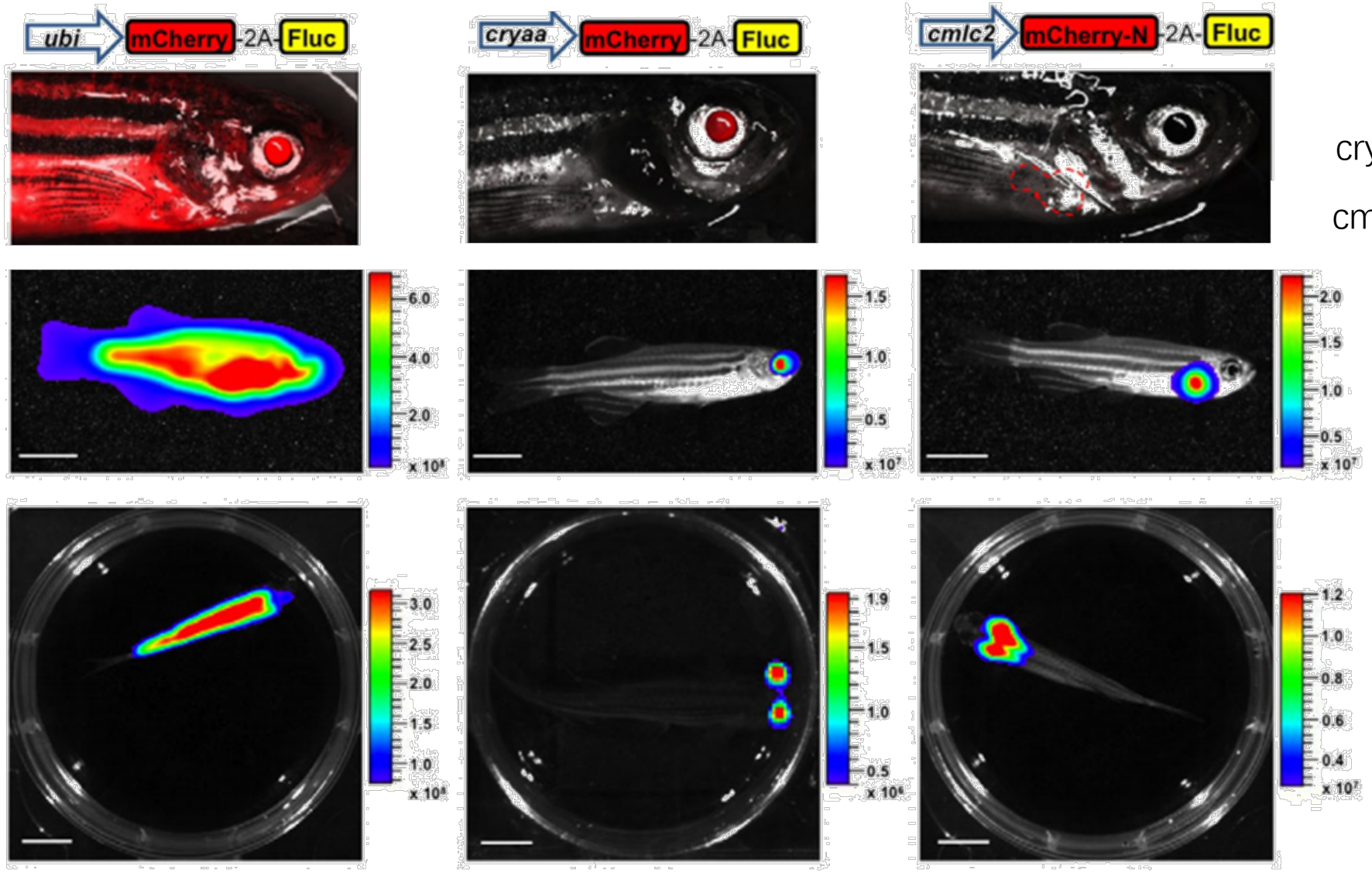


- ***Antrodia Camphorata*** (牛樟芝) is a unique mushroom from Taiwan that is said to have medicinal properties
- This study highlights the ability of *A. Camphorata to* inhibit LPS-induced inflammation in NFκB-luc mice, in vivo (left) and ex vivo (right)

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

Xing.Huang@perkinelmer.com

检测启动子活性



ubi: 泛素启动子

cryaa: α crystallin 晶体蛋白启动子

cmlc2: 心肌肌球蛋白启动子

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