PerkinElmer
For the Better

HUMAN HEALTH

IN VIVO IMAGING

IVIS®小动物活体影像技术原理及应用分享

张建　活体影像技术支持

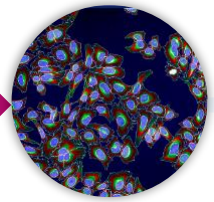
Jian.zhang@perkinelmer.com

PerkinElmer 生命科学领域解决方案

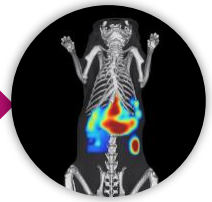
整合的解决方案，贯穿转化医学的所有环节

基因分析

分子检测

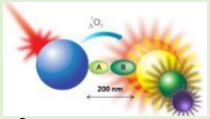


细胞成像



活体影像

核酸提取



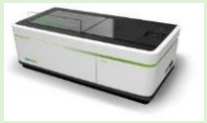
cisbio ALPHAPlex

ATPlite

高通量多标记检测

Spotfire® TIBCO Software

Harmony® 活细胞成像



高内涵细胞成像

小动物活体成像

服务于国内
众多科研院所、高校及企业

帮助中国科学家在顶尖
刊物发表**数万**余篇重要论文，
助力生命科学前沿探索

仪器 试剂 软件

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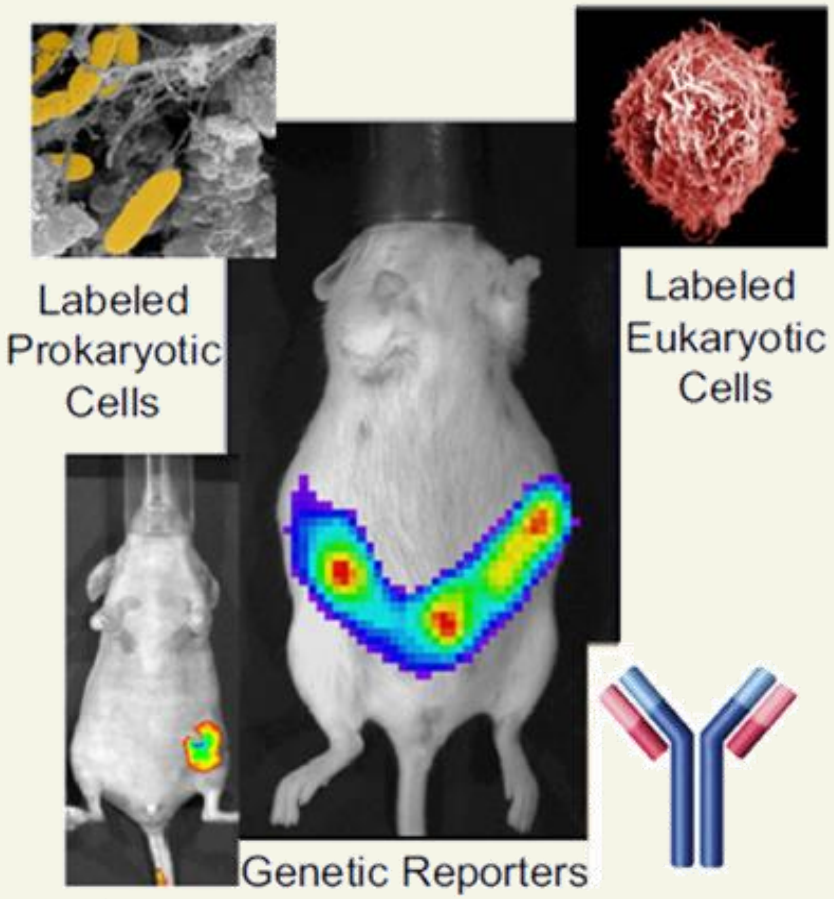
活体光学成像——技术核心

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。

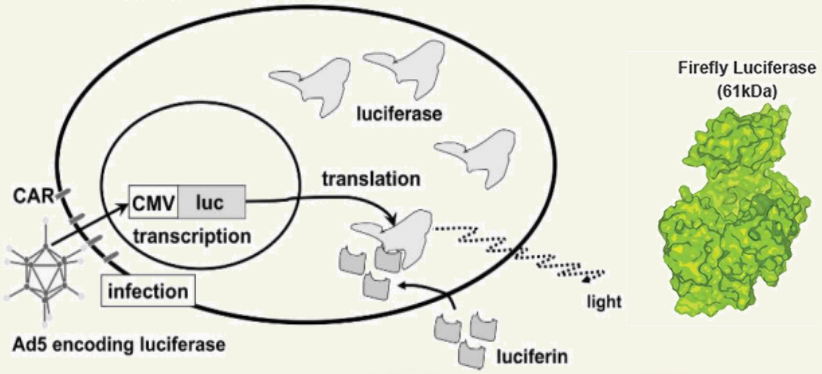
光学标记

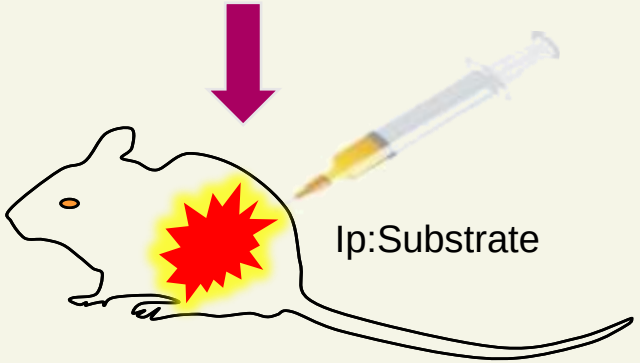


成像技术及设备

活体光学成像——标记方式

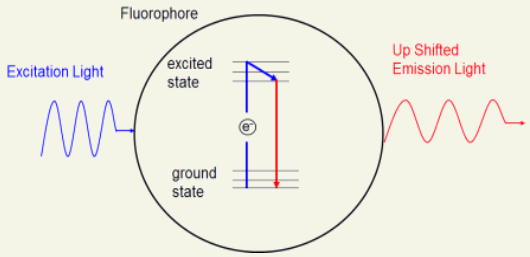
Bioluminescence

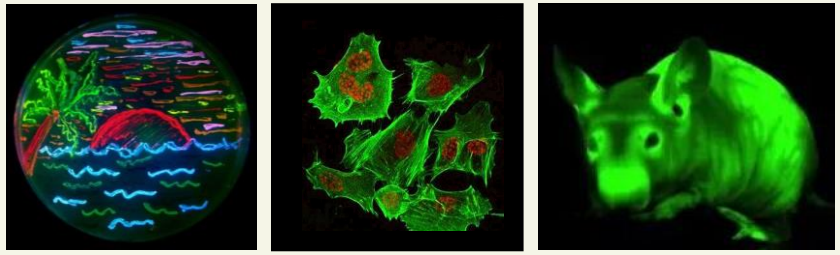


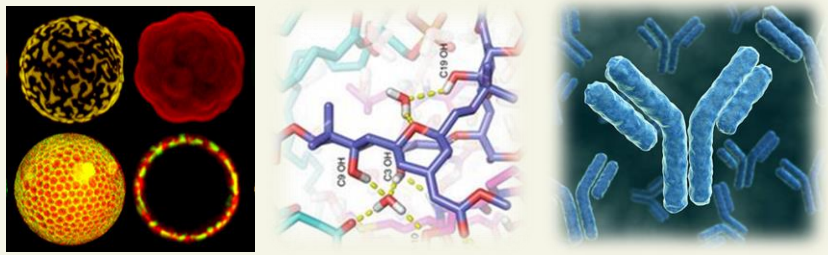


萤光素酶
+
底物

Fluorescence



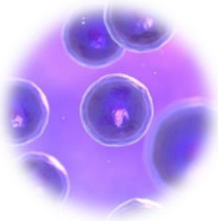
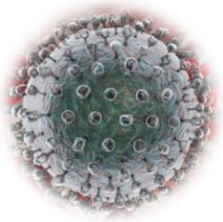


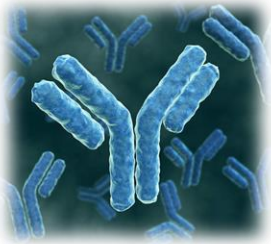
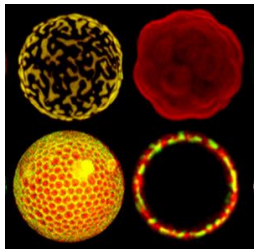
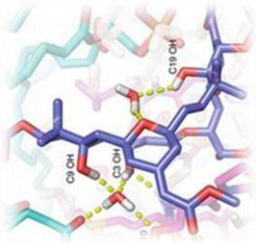
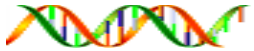


荧光分子
+
激发

标记方式-小结

HUMAN HEALTH | ENVIREMENTAL HEALTH

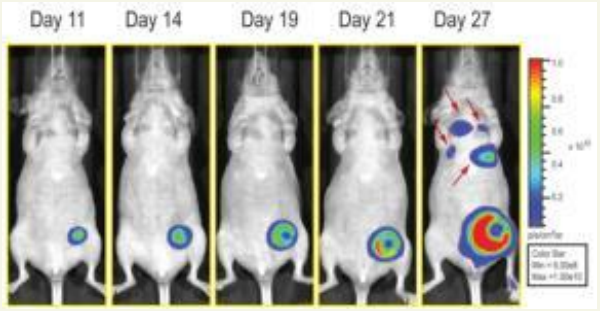
检测对象	标记方式
	荧光素酶（长期） 荧光蛋白（长期） 脂溶性荧光染料（短期） 荧光探针
	荧光素酶（长期） 荧光蛋白（长期） 荧光探针
	荧光素酶 （Nluc/Gluc）

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 荧光蛋白-膜上表达 活化荧光染料-膜上蛋白共价结合
	活化荧光染料-共价结合、从头合成
	活化荧光染料-共价结合

应用概述

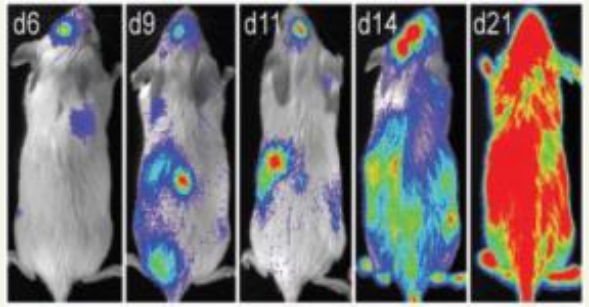
HUMAN HEALTH | ENVIREMENTAL HEALTH

监测疾病的发生发展及治疗



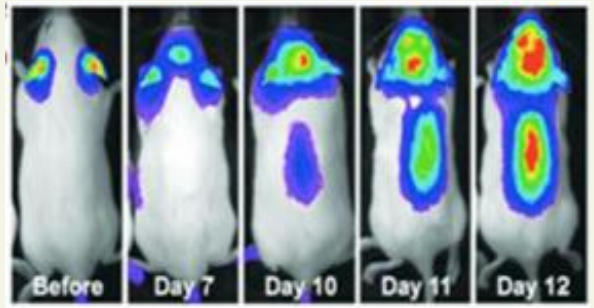
- 癌症
- 免疫性疾病
- 感染性疾病
- 代谢性疾病
- 心血管疾病
- 神经疾病

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



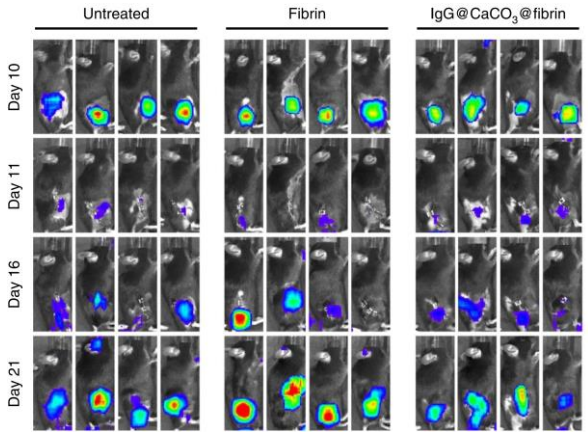
- 基因与疾病关系
- 疾病信号通路
- 细胞因子作用

肿瘤——模型的构建

HUMAN HEALTH | ENVIREMENTAL HEALTH

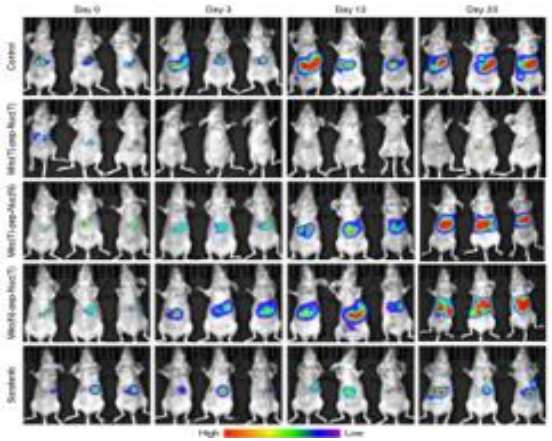
原位瘤模型

皮下黑色素瘤



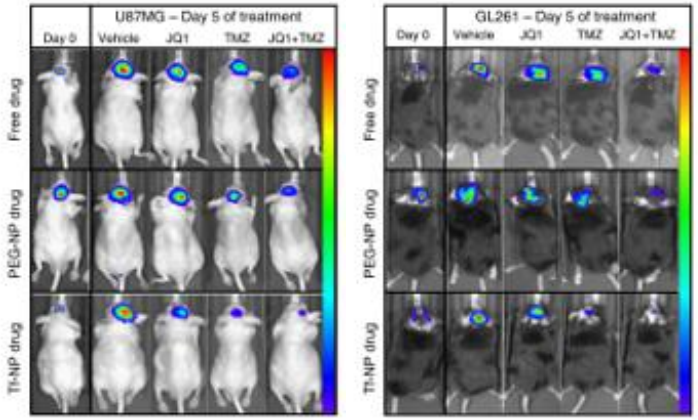
Nat. Nanotech. 2019,14: 89

原位肝肿瘤



Nat. Commu. 2019,10: 3051

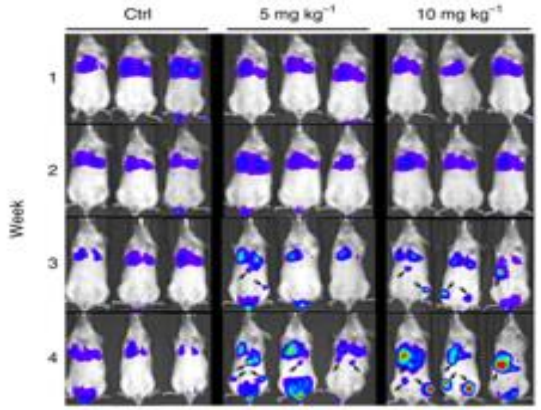
脑胶质瘤



Nat. Commu. 2018,9: 1991

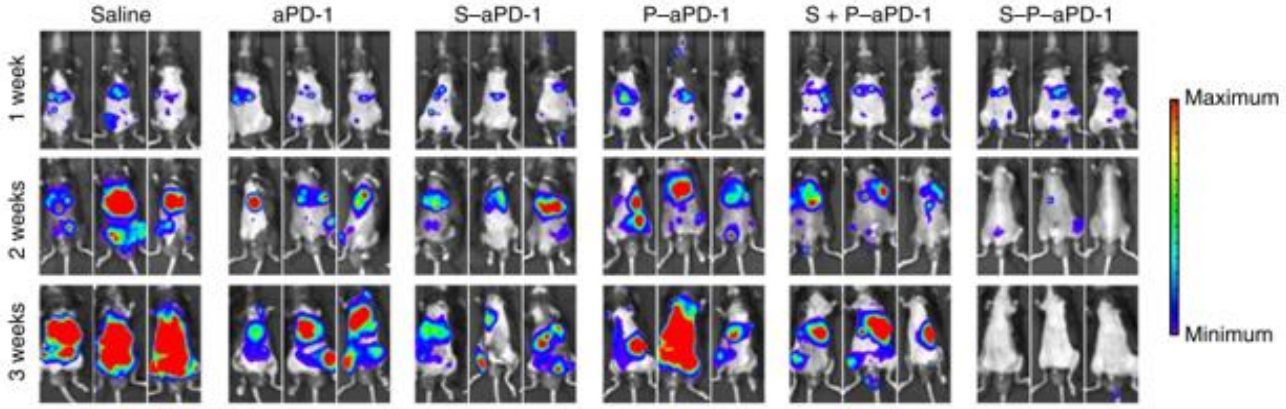
转移瘤模型

肺部转移



Nat. Nanotech. 2019,14: 279

急性骨髓白血病

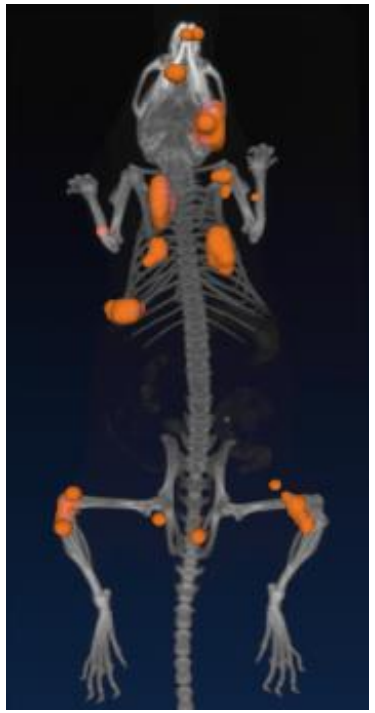


Nat. Biomed. Eng. 2019,14: 279

肿瘤——模型的构建

3D bioluminescent were co-registered with the the subject’s skeletal anatomy

Day17



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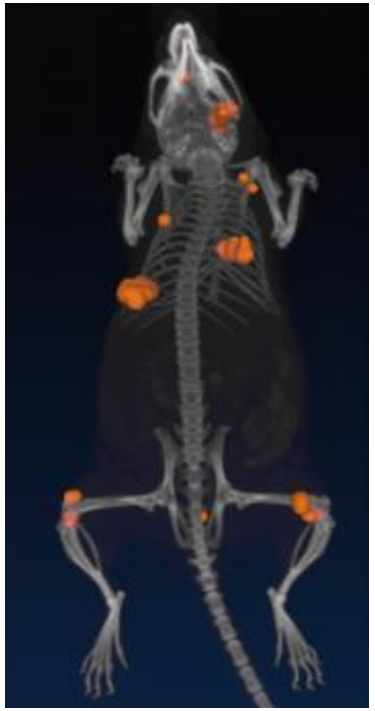

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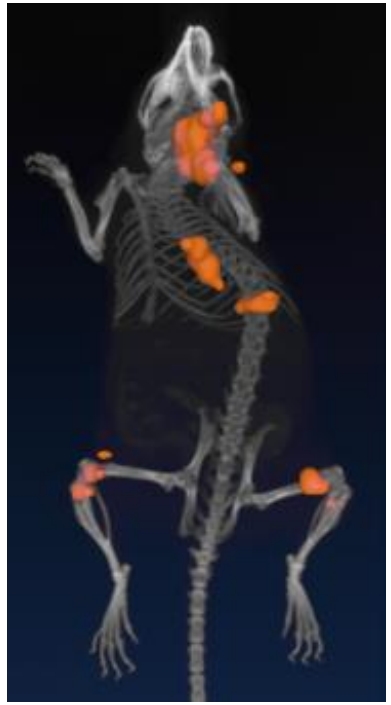
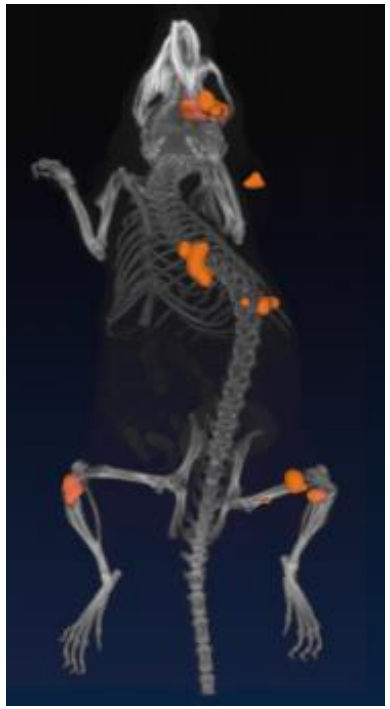
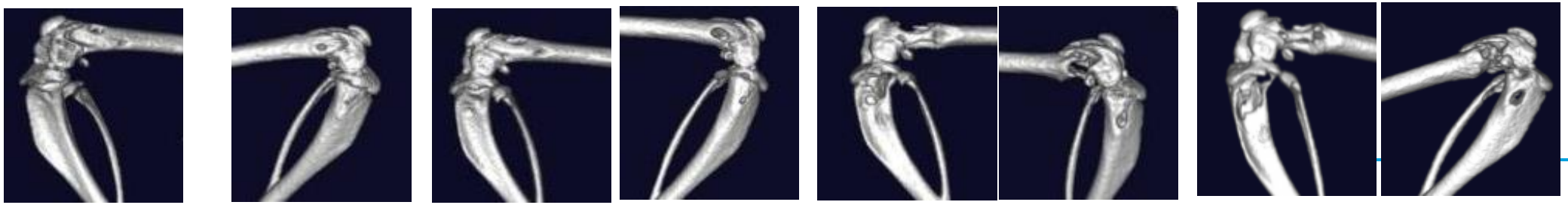


Day 31

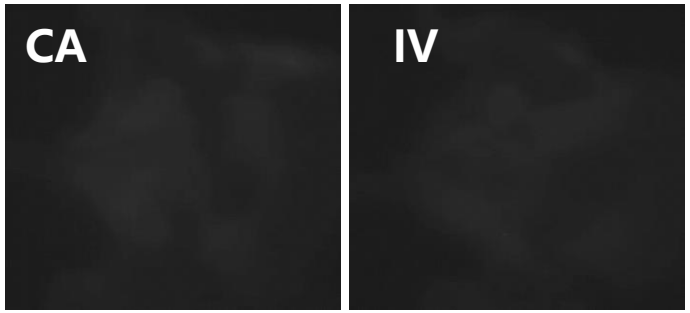


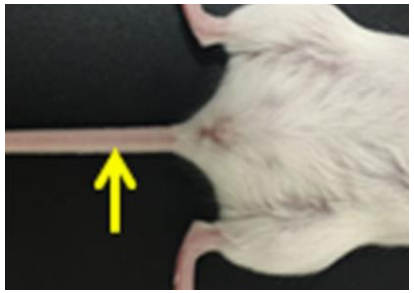


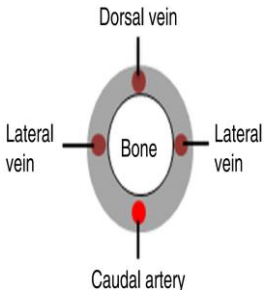
肿瘤发生发展——肿瘤研究模型的构建

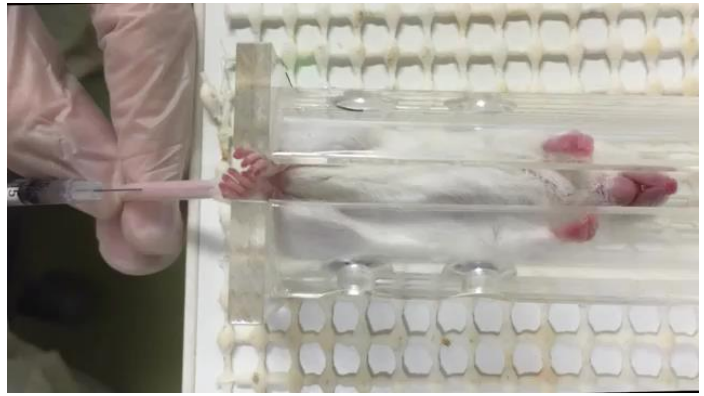
HUMAN HEALTH | ENVIREMENTAL HEALTH

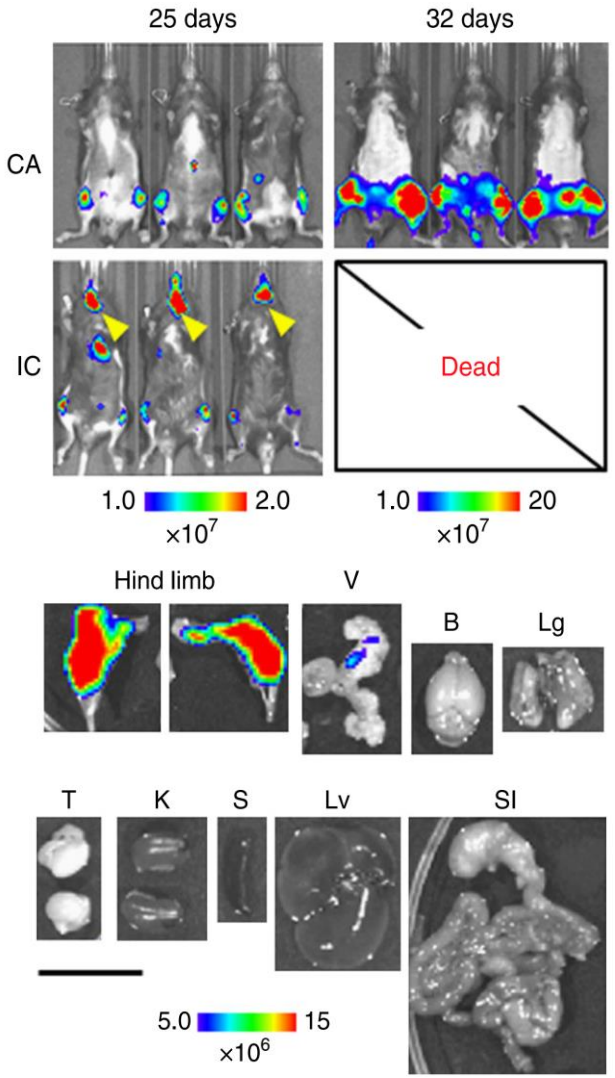
尾动脉注射肿瘤细胞构建骨转移模型

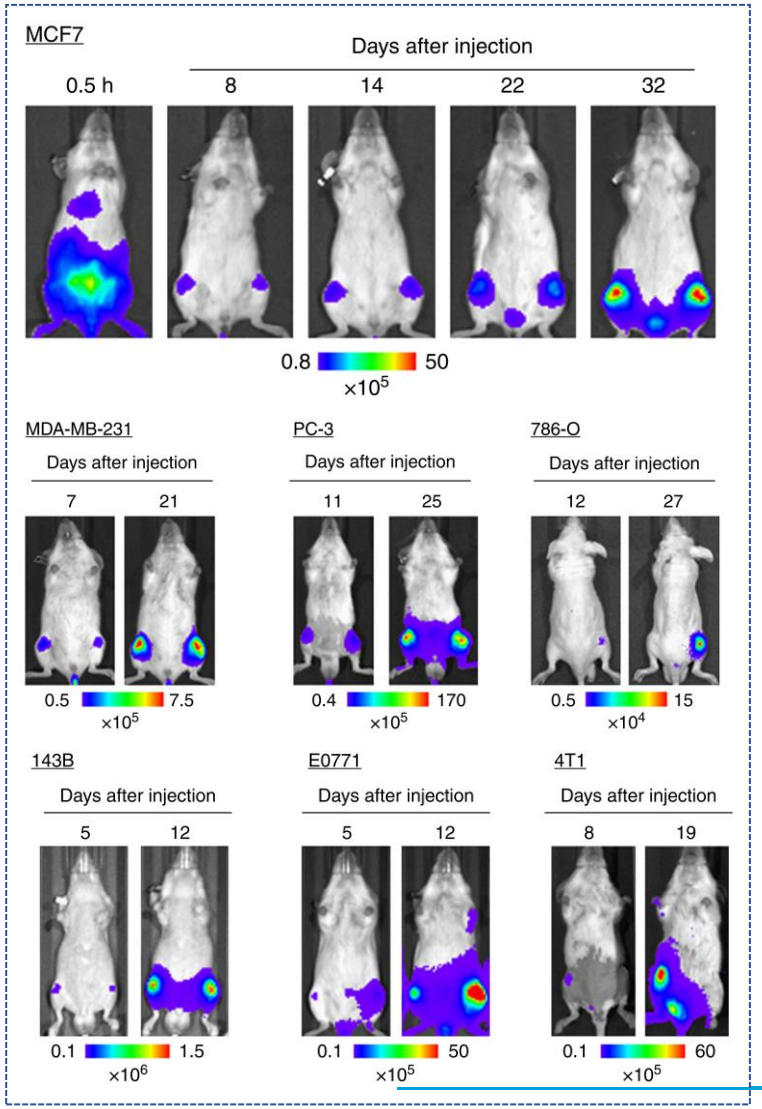








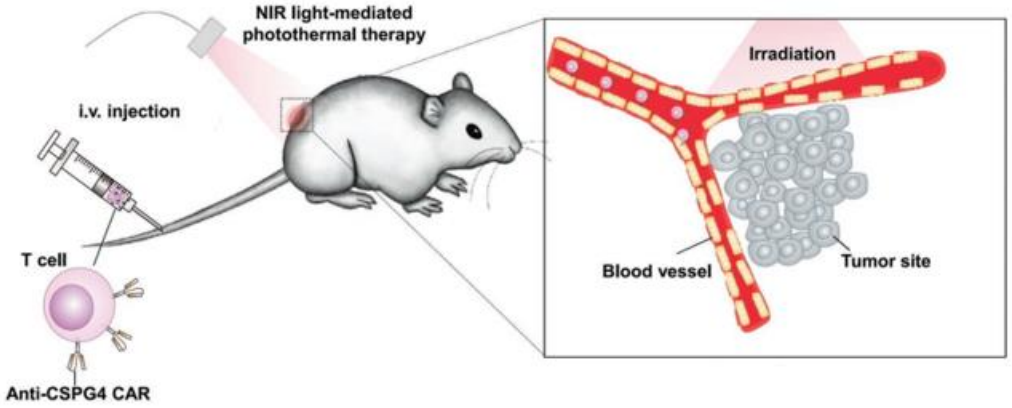




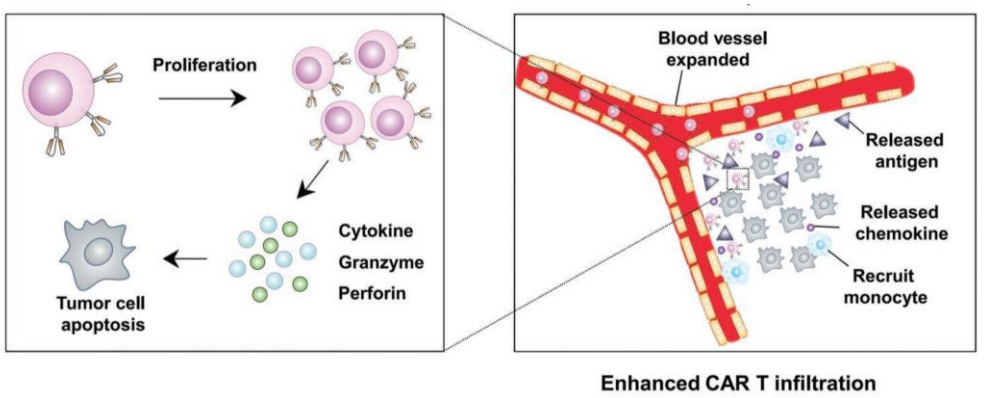
Nature Biomedical Engineering 2019, 3: 452-456

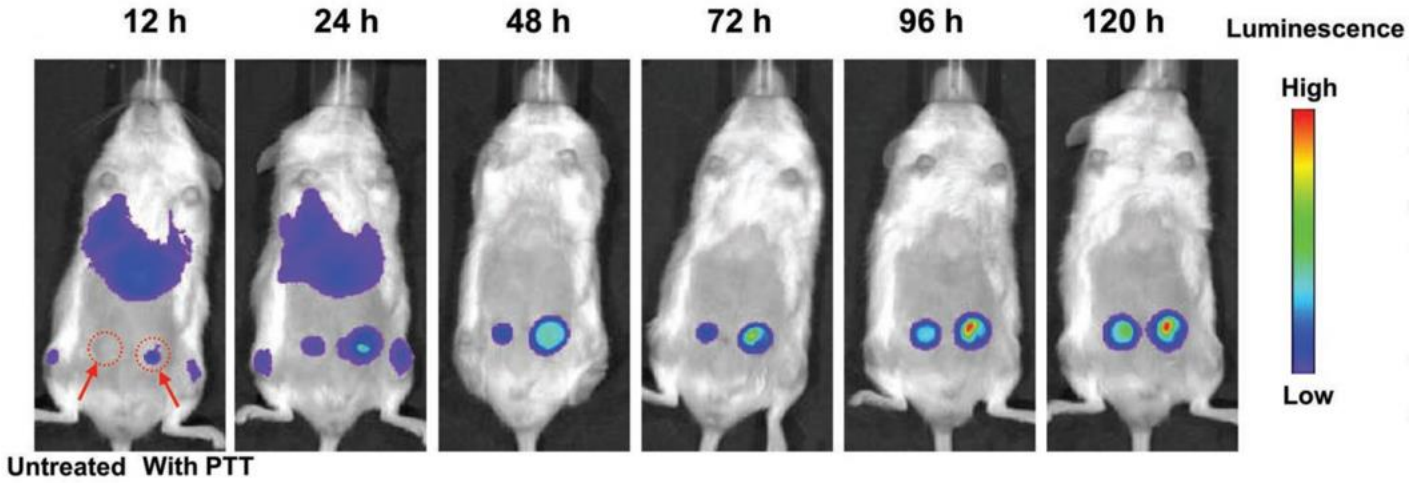
肿瘤治疗----CAR-T治疗

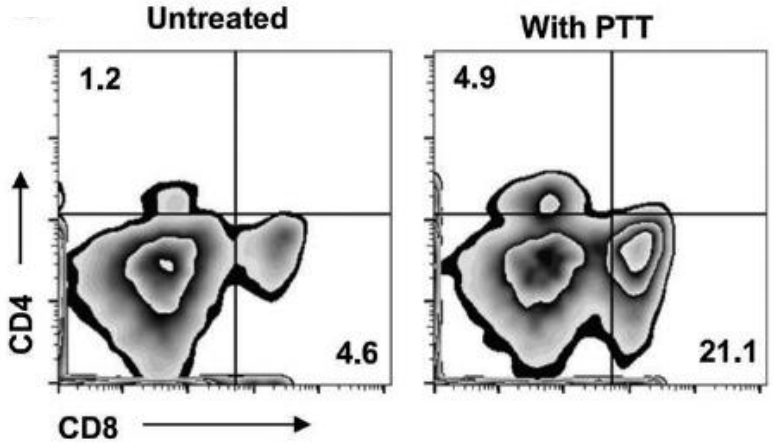
CAR-T细胞靶向肿瘤



After PTT



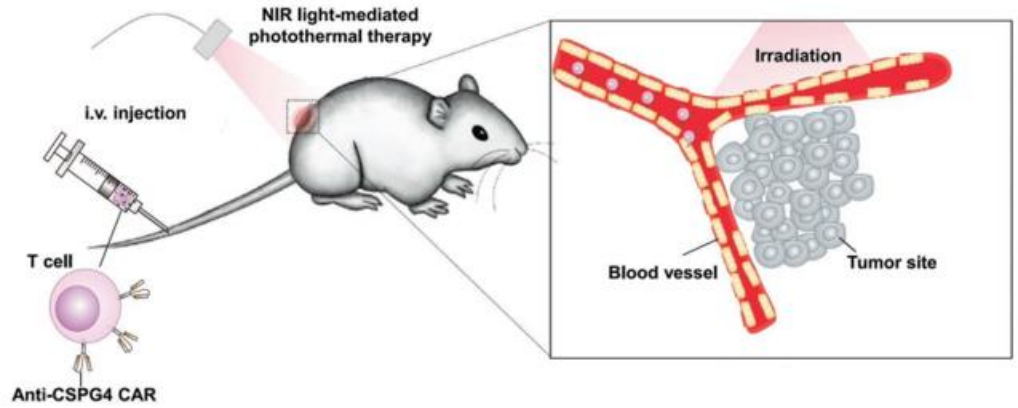




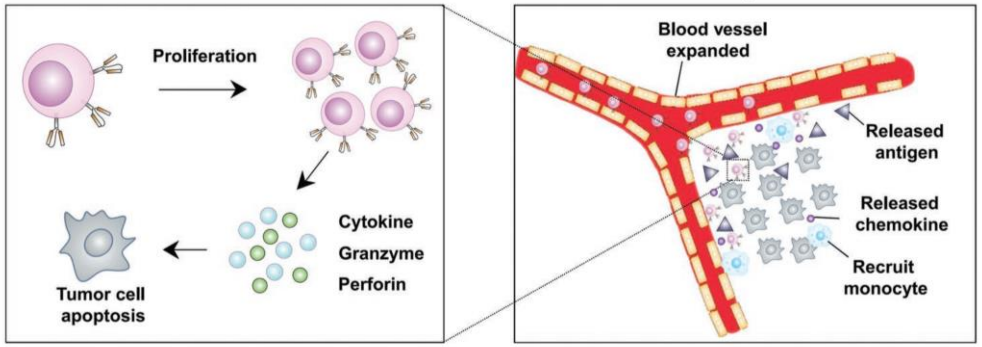
Adv. Mater. 2019, 1900192

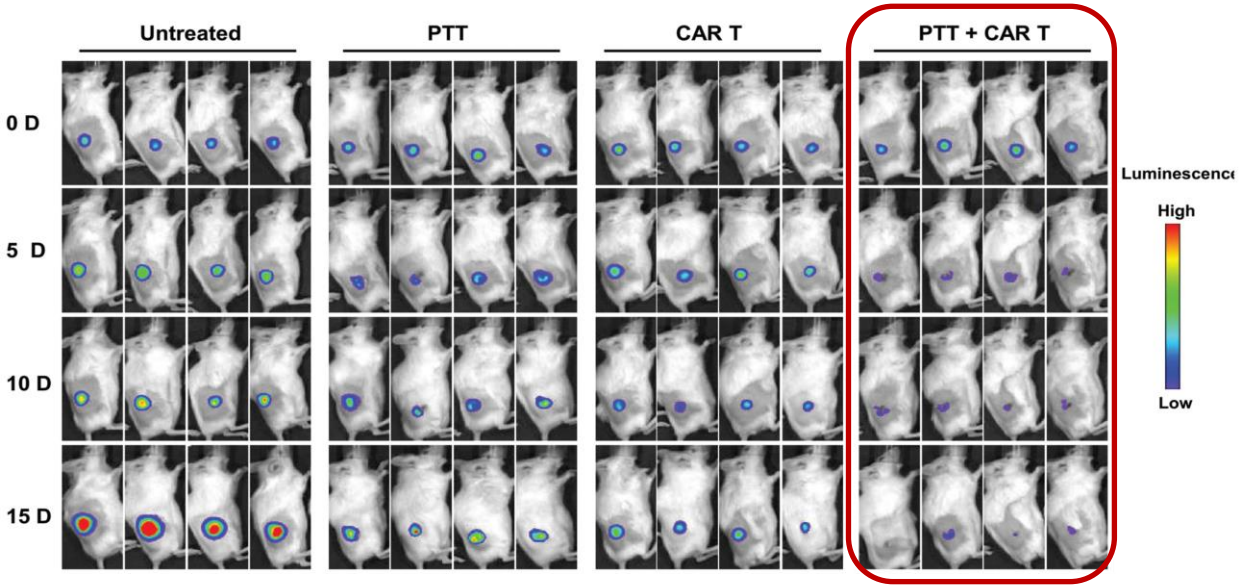
肿瘤治疗----CAR-T治疗

CAR-T 细胞结合光热作用（PTT）治疗实体肿瘤

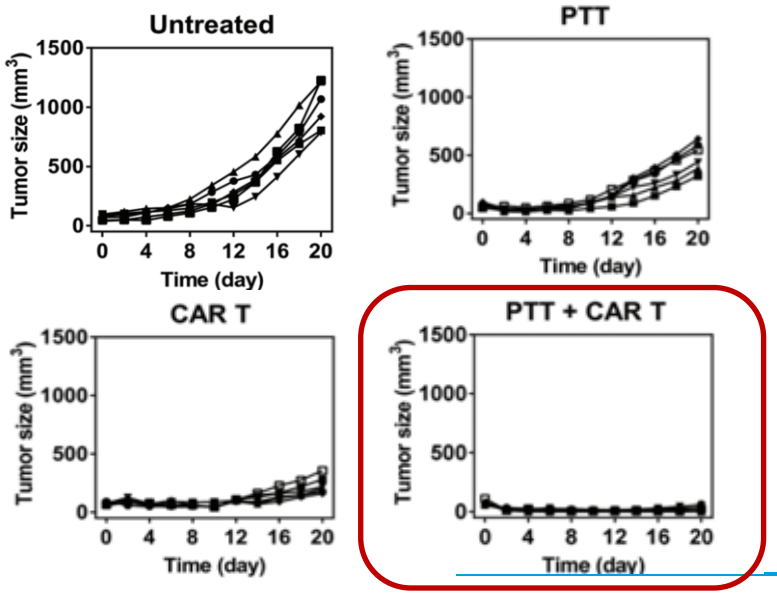


After PTT





Enhanced CAR T infiltration

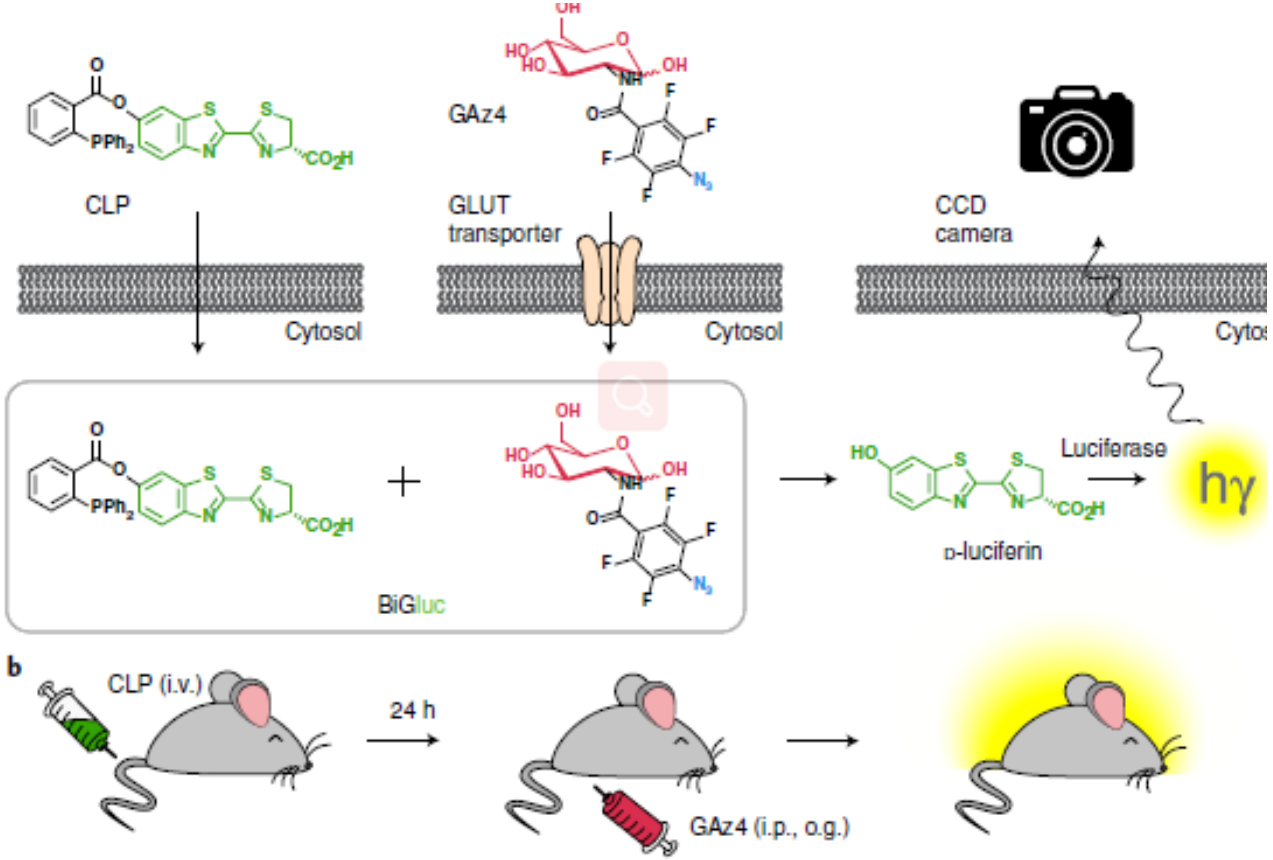


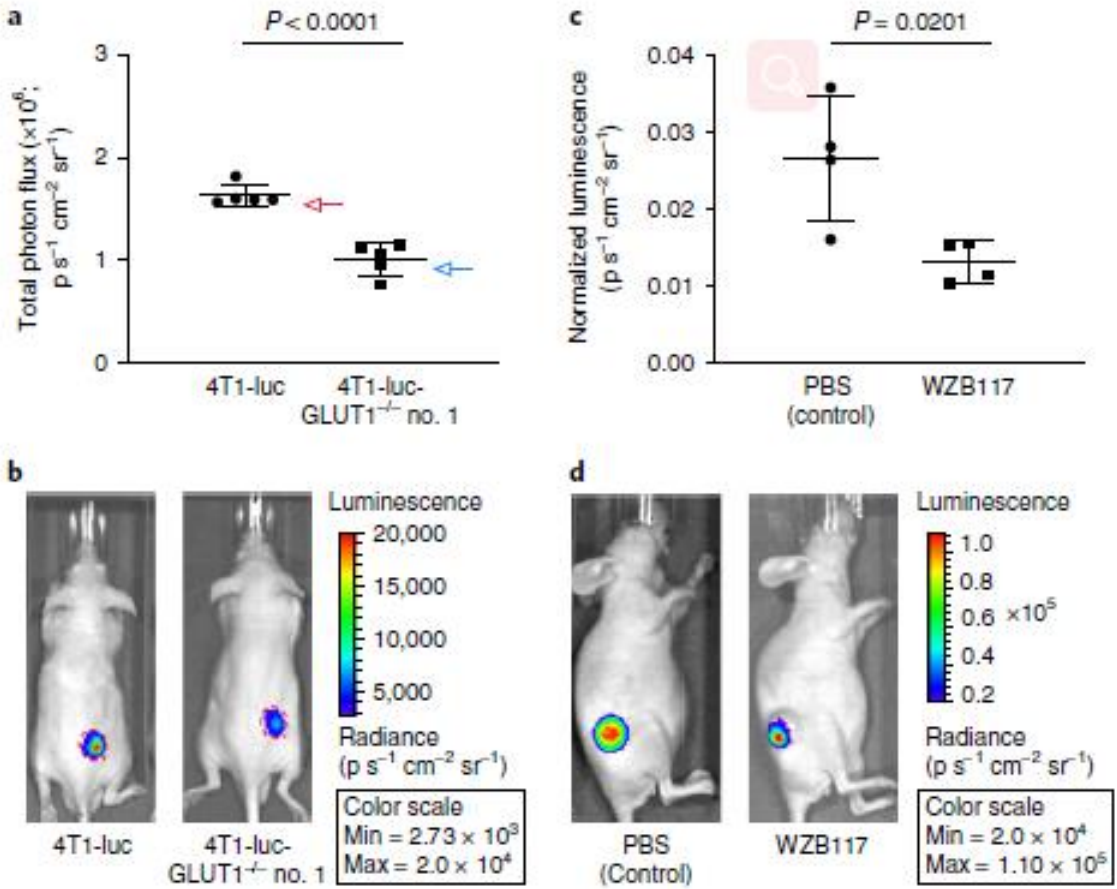
Adv. Mater. 2019, 1900192

肿瘤——肿瘤代谢

nature methods

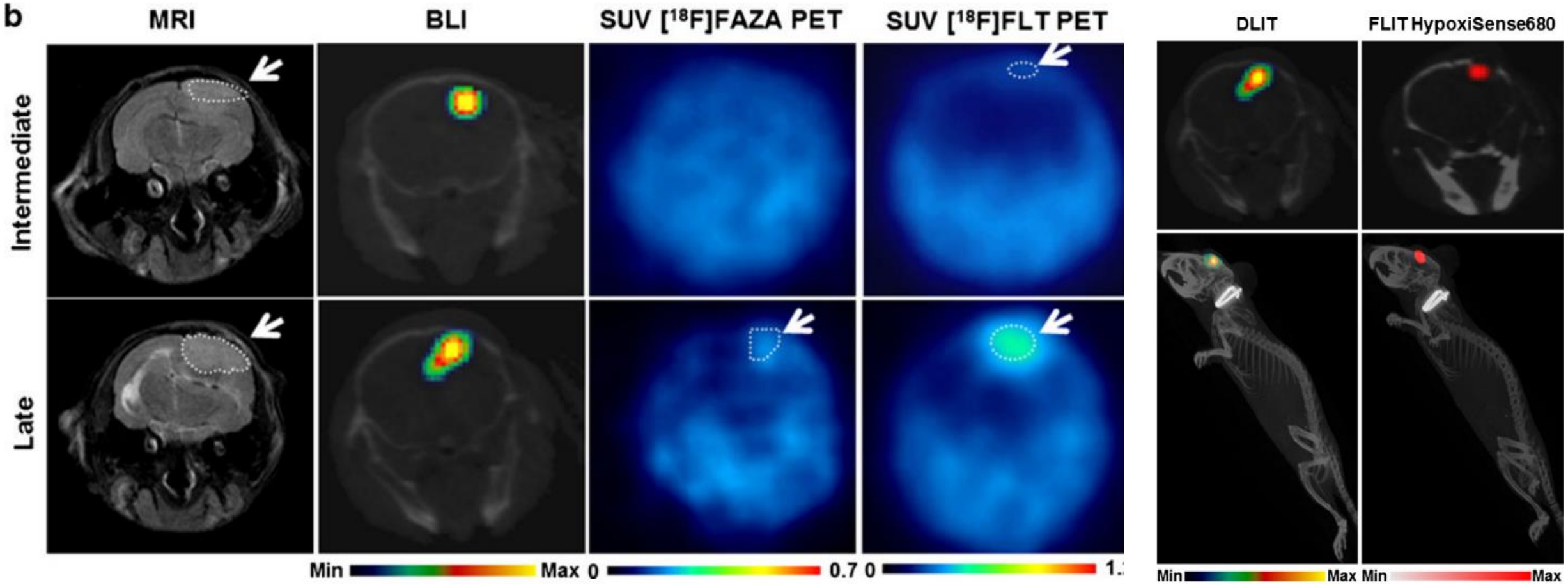
Bioluminescent-based imaging and quantification of glucose uptake in vivo





肿瘤代谢:　萤光素三芳基膦　葡萄糖叠氮化物

肿瘤——微环境

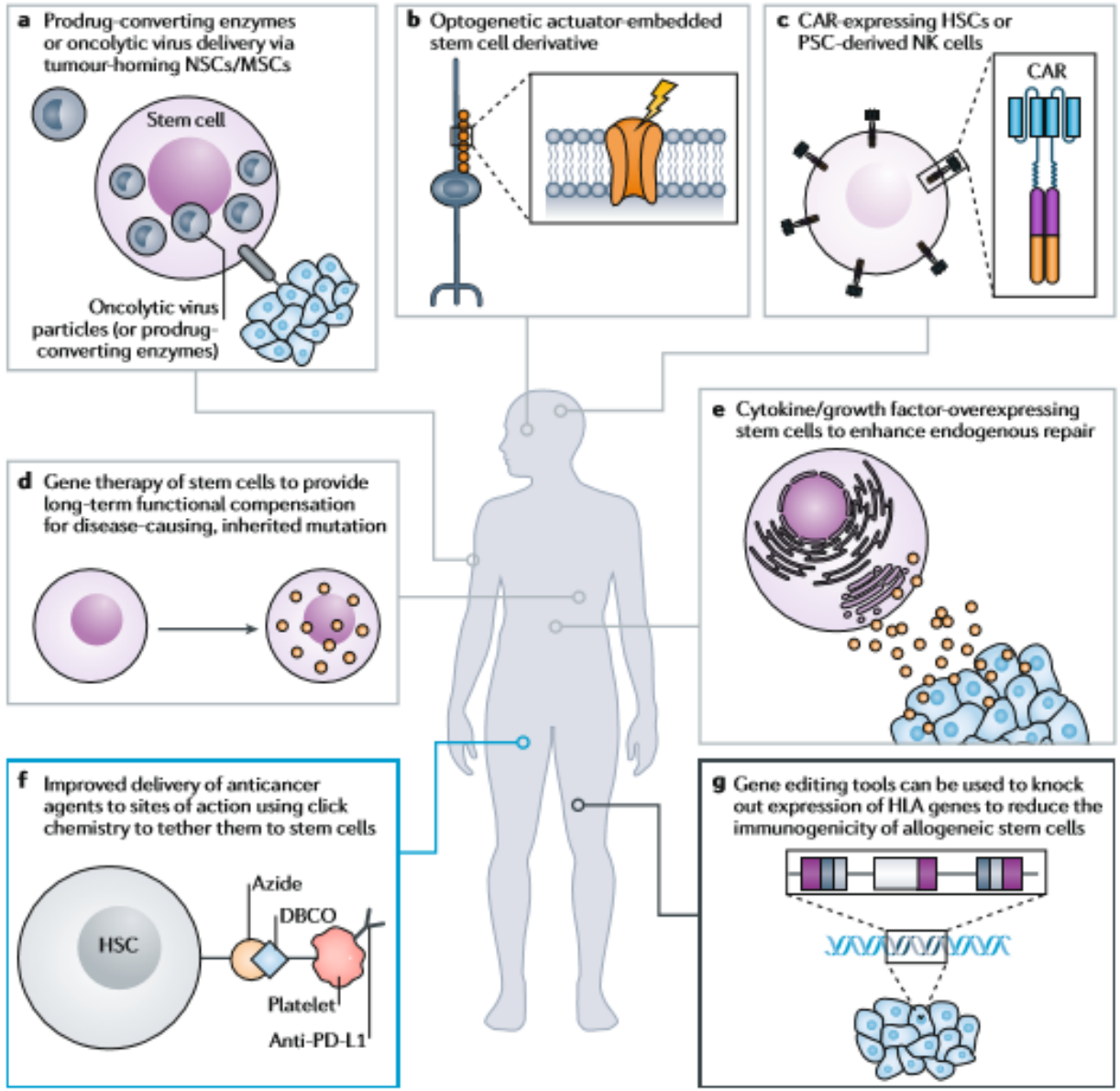


Lo Dico, A., *et al.* Mol Imaging Biol (2014) 16: 210.

缺氧环境中高表达的碳酸酐酶9

干细胞治疗

CLICK TO ADD CAPTION TEXT



Virus as a key tool
Click chemistry
Nuclease or AAV-based gene editing

病毒

肿瘤归巢载药

光遗传增加活性

肿瘤免疫应用：HSC-CAR, PSC-derived NK

基因治疗:治疗遗传性疾病

增强组织修复：表达细胞因子

点击化学

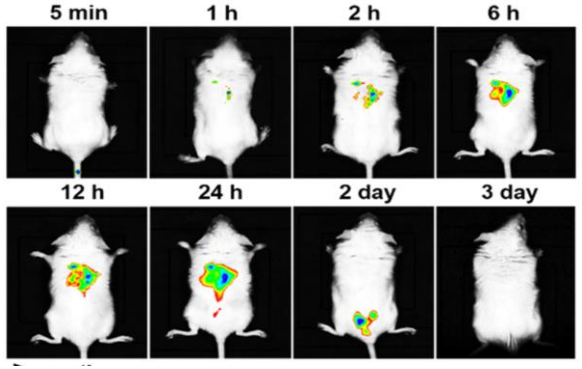
将抗癌药物捆绑在干细胞上，提高了抗癌药物的作用位点

基因编辑

敲除HLA基因的表达，降低异体干细胞的免疫原性

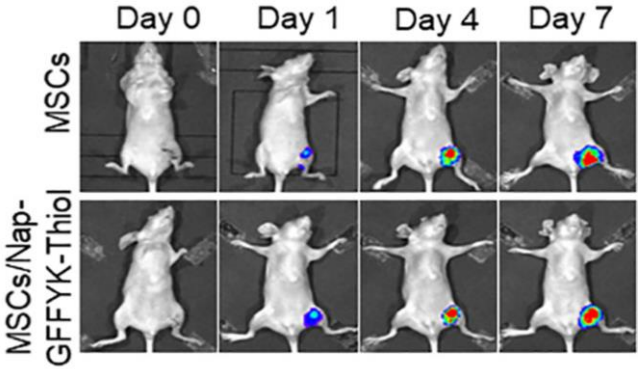
干细胞损伤治疗

肝损伤



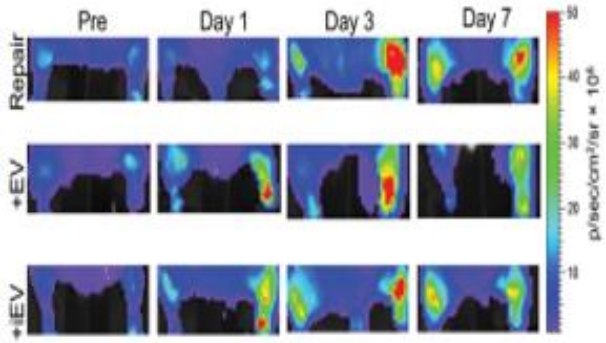
ACS Appl. Mater. Interfaces 2019, 11, 8763

下肢缺血

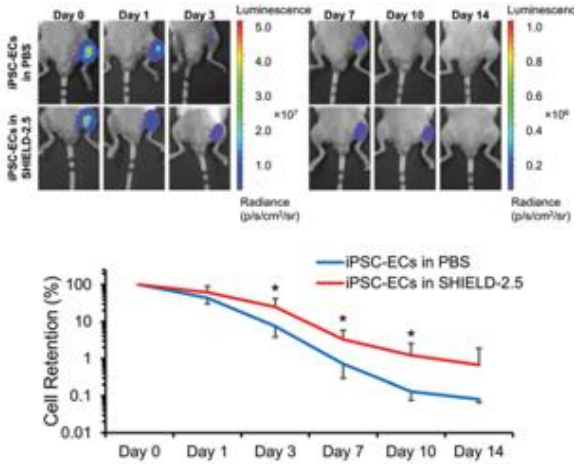


Acta Biomaterialia 2019, 85: 94

肌腱损伤

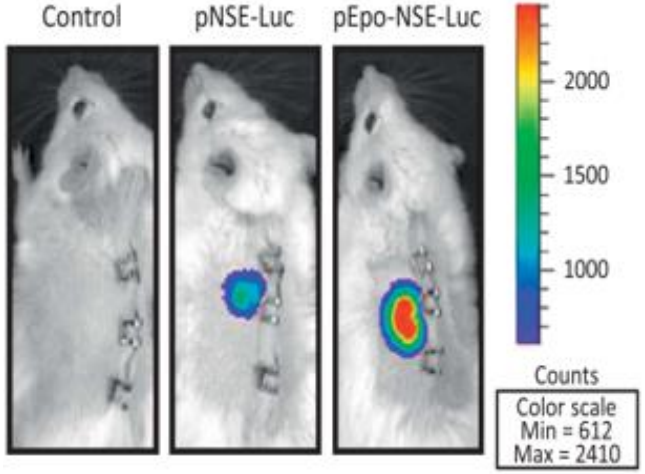


外周动脉



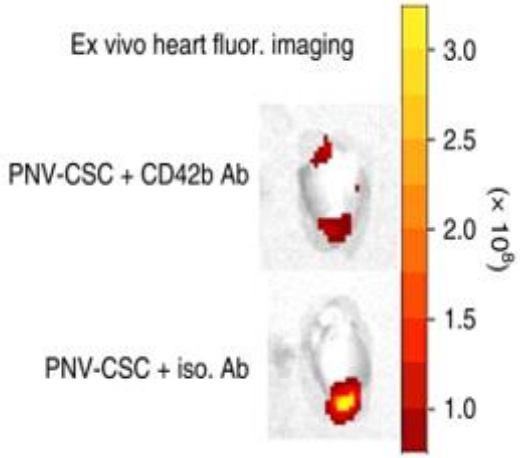
Biomater. Sci., 2018, 6, 614

脊柱损伤



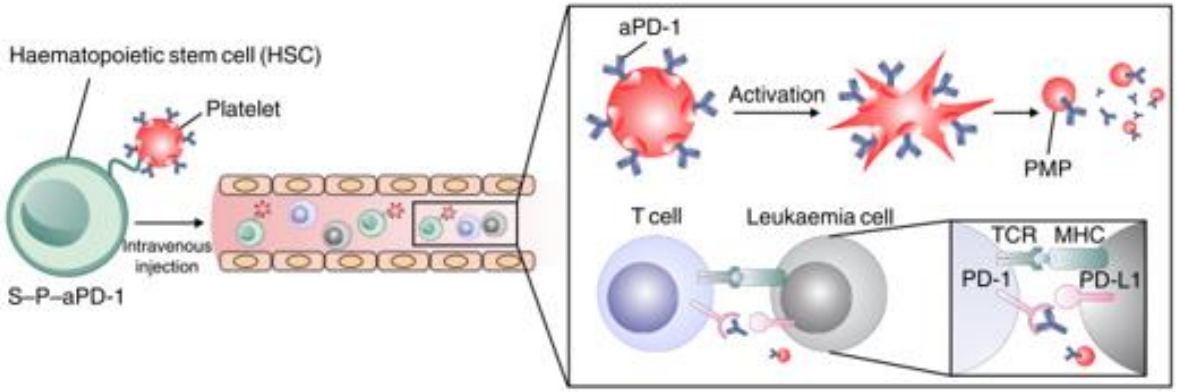
Gene Therapy 2018: 27

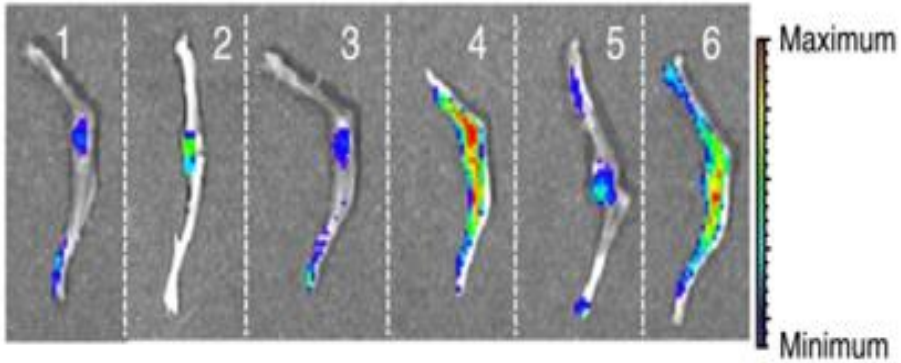
心脏损伤

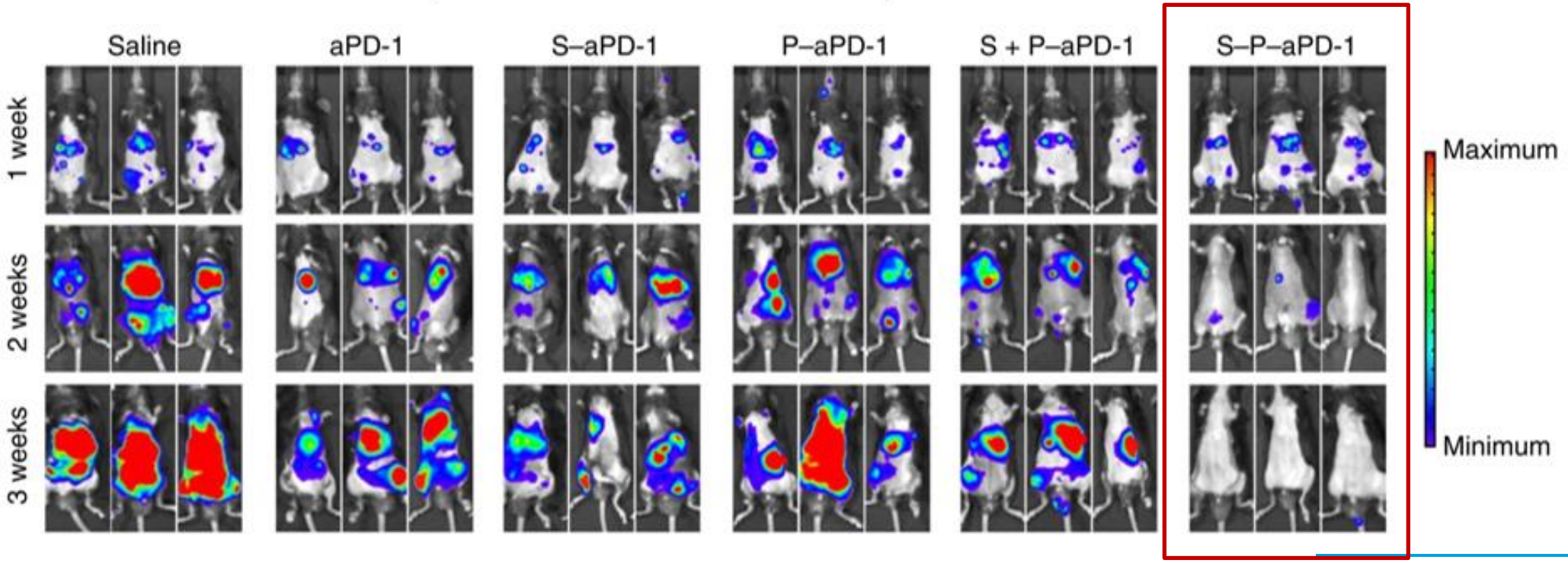


Nat. Biomed. Enginee. 2018, 2: 17

干细胞治疗-肿瘤治疗

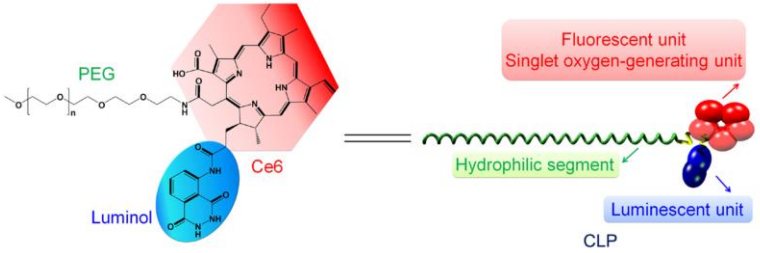


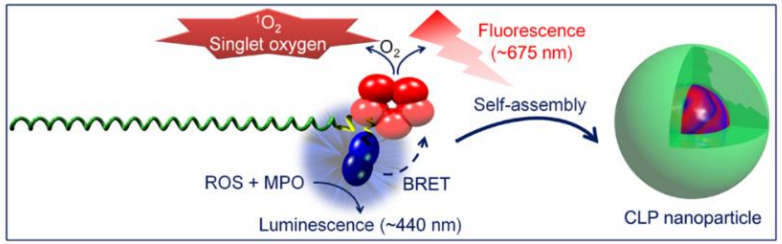


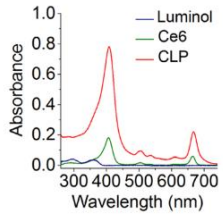


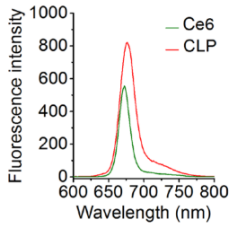
Nature Biomedical engineering 2018, 2: 831-840

炎症监测





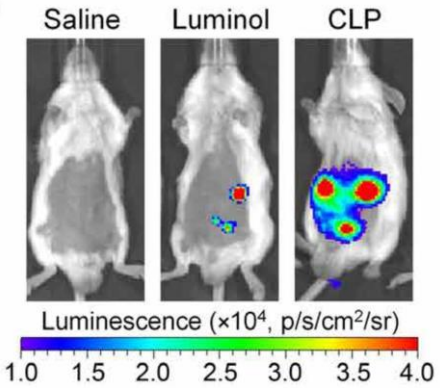




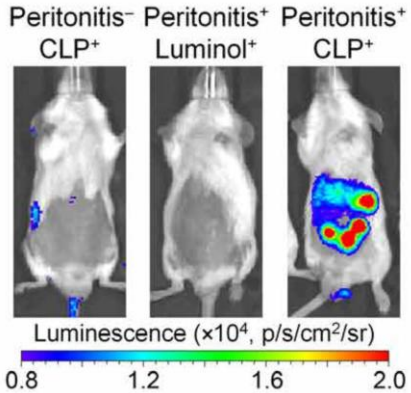
腹膜炎模型

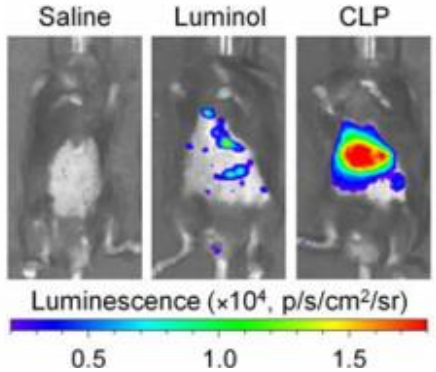
急性肝损伤模型

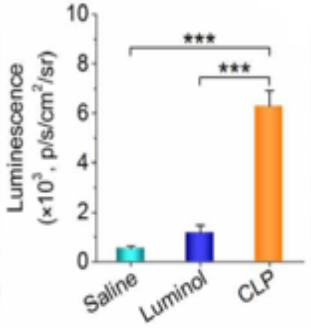
腹腔注射



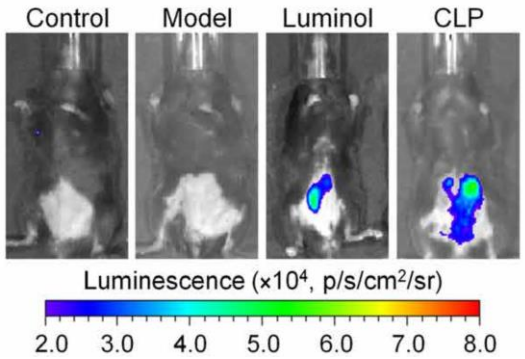
静脉注射

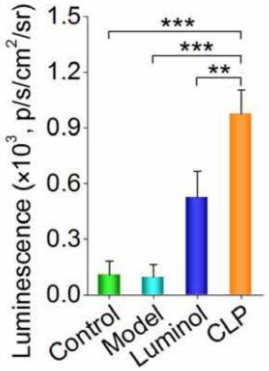


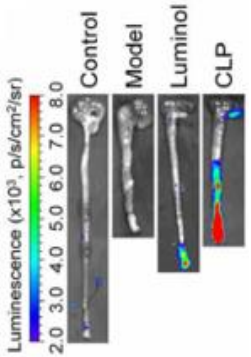


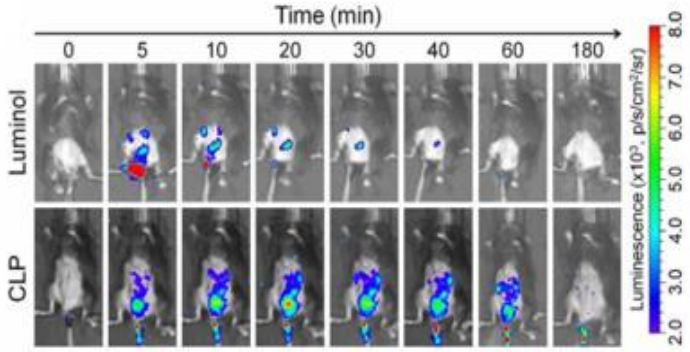


结肠炎模型





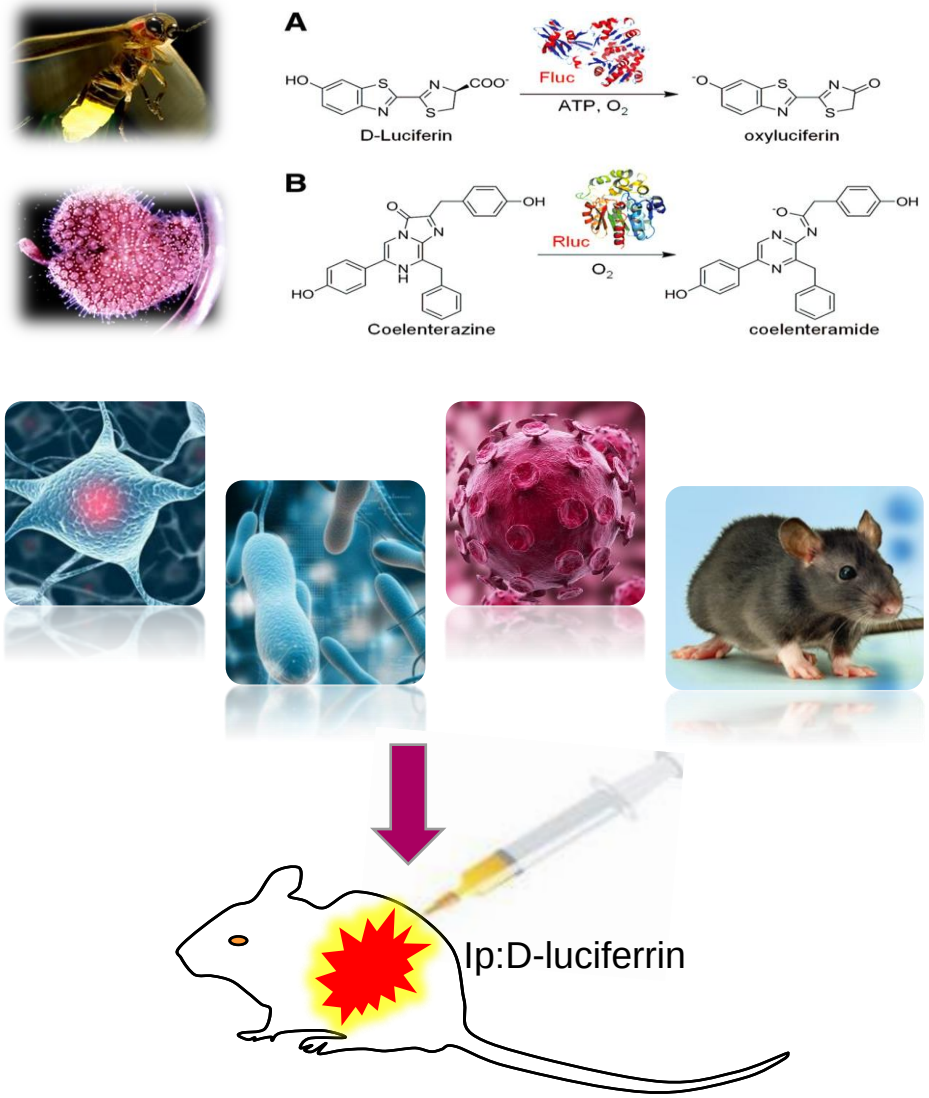


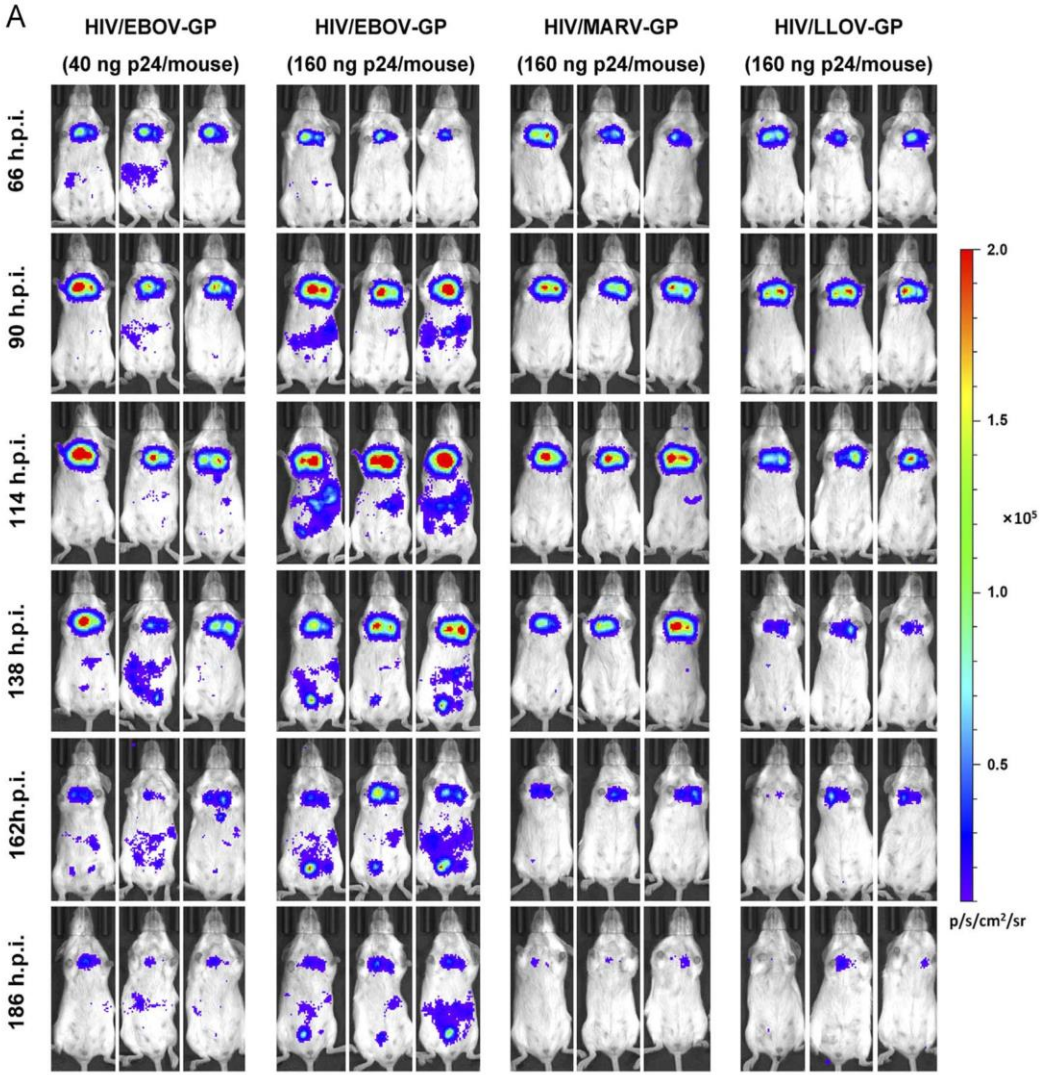


Xu et al., Science Advances 2019; 5：2953

病毒研究-监测和治疗

HUMAN HEALTH | ENVIREMENTAL HEALTH



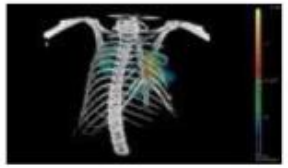


Acta pharmaceutica sinica B, 2018, 8(2): 200-208

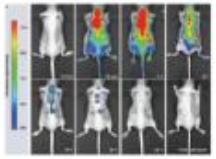
活体光学成像——应用概述

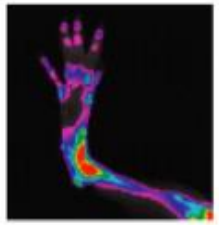
HUMAN HEALTH | ENVIREMENTAL HEALTH

肿瘤癌症



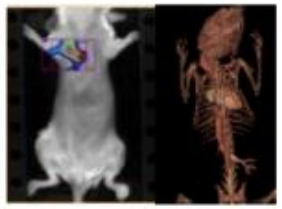
纳米材料研究





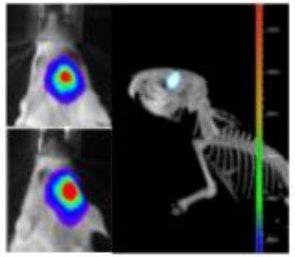
炎症

心血管疾病

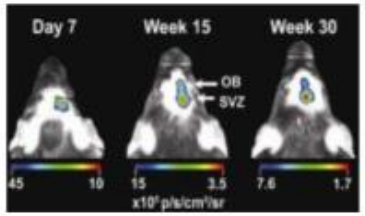


药物研究

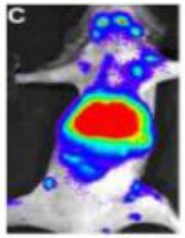
神经科学



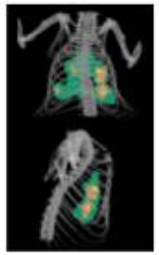
干细胞研究



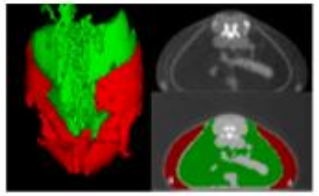
免疫和移植生物学



传染性疾病



代谢研究

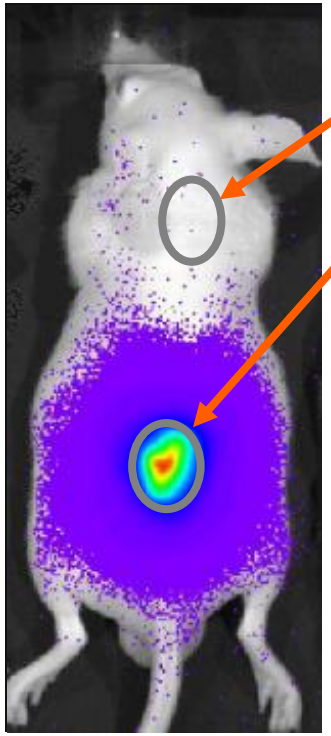


实验注意事项

- 成像模式选择
- 荧光成像
- 生物发光成像

成像模式选择——生物发光 vs 荧光

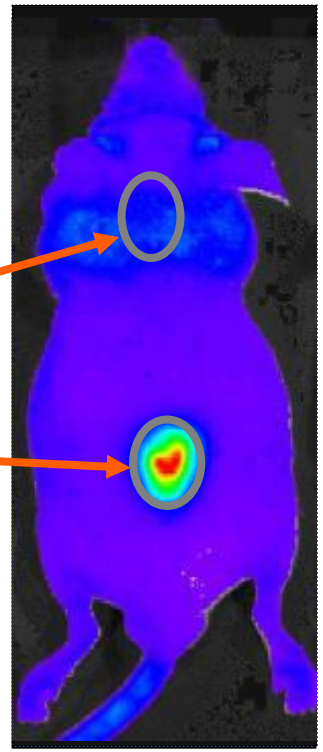
生物发光成像



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



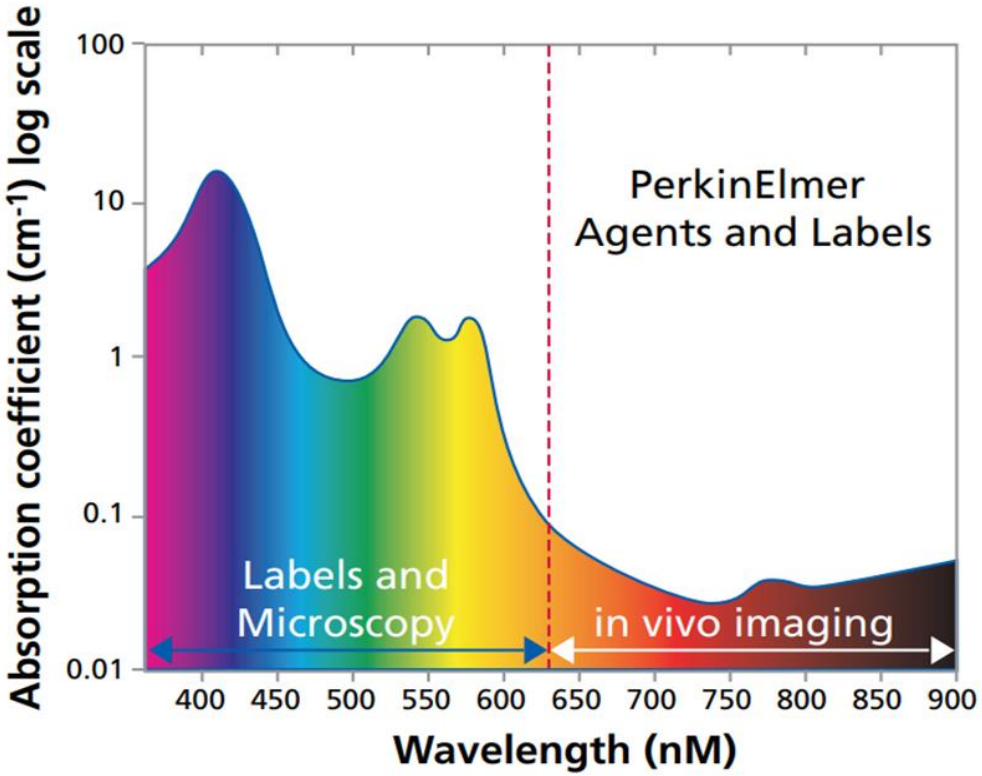
3×10^{6} PC3M-luc/DsRed cells injected s.c.

荧光成像灵敏度通常比生物发光成像低1000倍

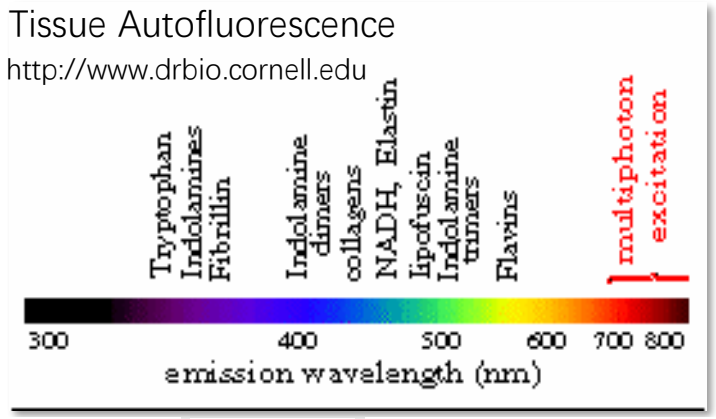
T.L. Troy, et al. (2004). Molecular Imaging 3:9-23.

荧光波长选择—透过率和背景噪音

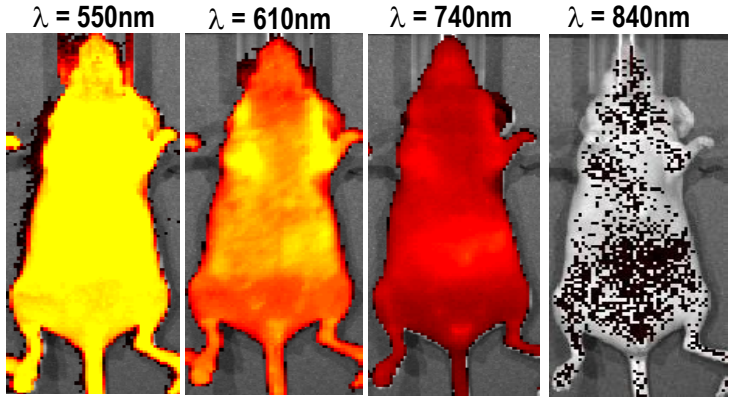
SNR= Signal / Autofluorescence



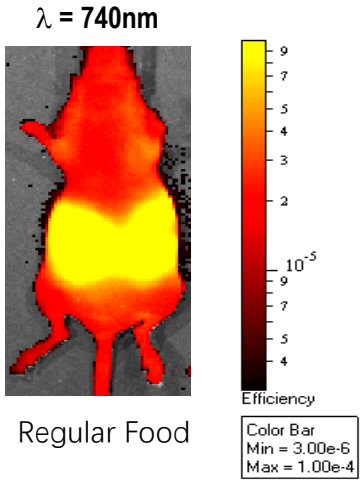
血红蛋白和其他机体组分强烈吸收可见光。
在近红外区域，组织吸收显著降低。



苜蓿



Alfalfa Free Rodent Food



Regular Food

荧光成像-不同类型荧光探针

PerkinElmer
For the Better

HUMAN HEALTH | ENVIREMENTAL HEALTH

Fluorescent Agents

MMPSense™ 680	Activatable	Red/NIR Imaging Agents
MMPSense™ 645/750 FAST	Activatable	Red/NIR Imaging Agents
ProSense® 680	Activatable	Red/NIR Imaging Agents
ProSense® 750EX	Activatable	Red/NIR Imaging Agents
ProSense® 750 FAST™	Activatable	Red/NIR Imaging Agents
Cat K 680 FAST™	Activatable	Red/NIR Imaging Agents
Cat B 680/750 FAST™	Activatable	Red/NIR Imaging Agents
Neutrophil Elastase 680 FAST™	Activatable	Red/NIR Imaging Agents
ReninSense 680 FAST™	Activatable	Red/NIR Imaging Agents
IntegriSense™ 645/680/750	Targeted	Red/NIR Imaging Agents
OsteoSense® 680EX/750EX/800	Targeted	Red/NIR Imaging Agents
Annexin-Vivo 750	Targeted	Red/NIR Imaging Agents
RediJect Bacteria Probe 750	Targeted	Red/NIR Imaging Agents
HypoxiSense™ 680	Targeted	Red/NIR Imaging Agents
FolateRSense™ 680	Targeted	Red/NIR Imaging Agents
BombesinRSense™ 680	Targeted	Red/NIR Imaging Agents
Transferrin-Vivo™ 750	Targeted	Red/NIR Imaging Agents
GastroSense™ 750	Physiologic	Red/NIR Imaging Agents
Genhance™ 680/750	Physiologic	Red/NIR Imaging Agents
Superhance™ 680	Physiologic	Red/NIR Imaging Agents
AngioSense® 680/750EX	Physiologic	Red/NIR Imaging Agents
AngioSPARK® 680/750	Physiologic	Red/NIR Imaging Agents
GFR-Vivo™ 680	Physiologic	Red/NIR Imaging Agents
VivoTag® 645/680/800	Labels	Red/NIR Imaging Agents
VivoTag®-S 680/750	Labels	Red/NIR Imaging Agents
VivoTag® 680/800 XL	Labels	Red/NIR Imaging Agents
Xenolight DiR	Labels	Red/NIR Imaging Agents
VivoTRACK™ 680	Labels	Red/NIR Imaging Agents

Applications

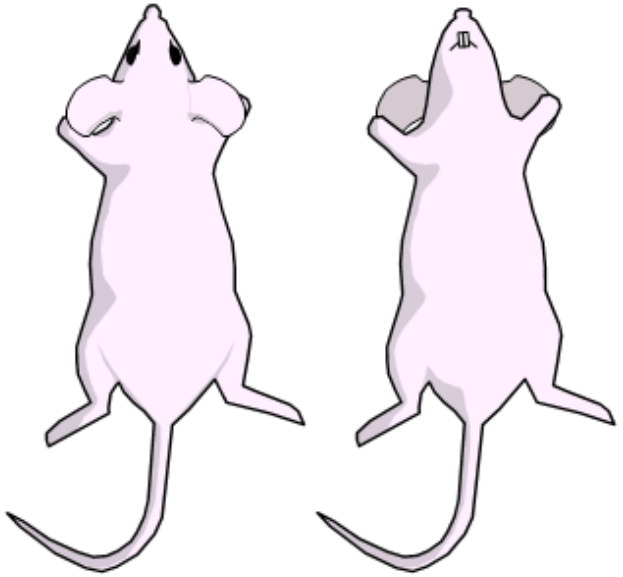
Cancer
- **Progression**
- **Treatment**
- **Biomarkers**

Inflammation
- Acute/Chronic
- Arthritis
- Pulmonary
- Other

Tox/Safety
- Liver
- Kidney
- Gastric

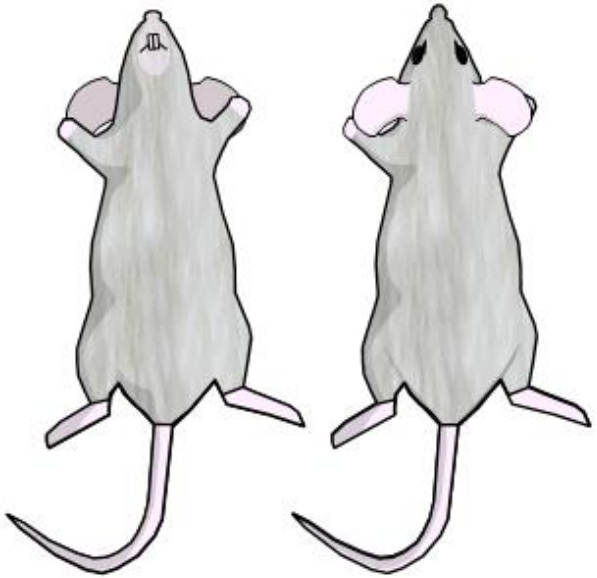
Pharmacology
- Biodistribution
- Pharmacokinetics

实验动物必须剃毛



Depilation not required

- Nude mice
- SKH1-E

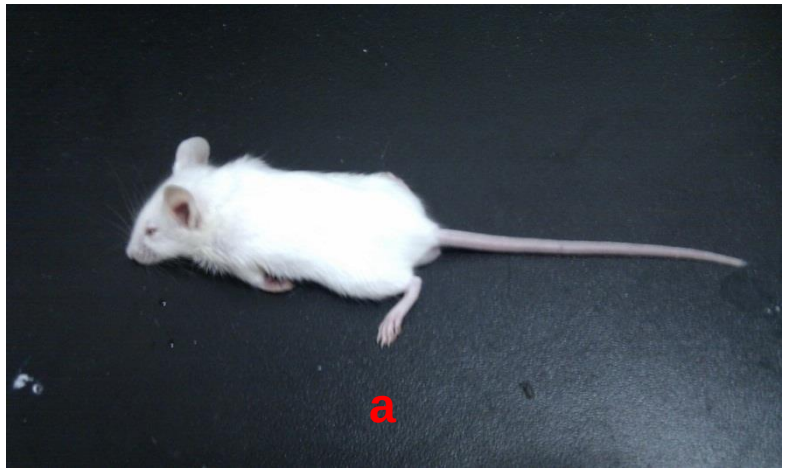


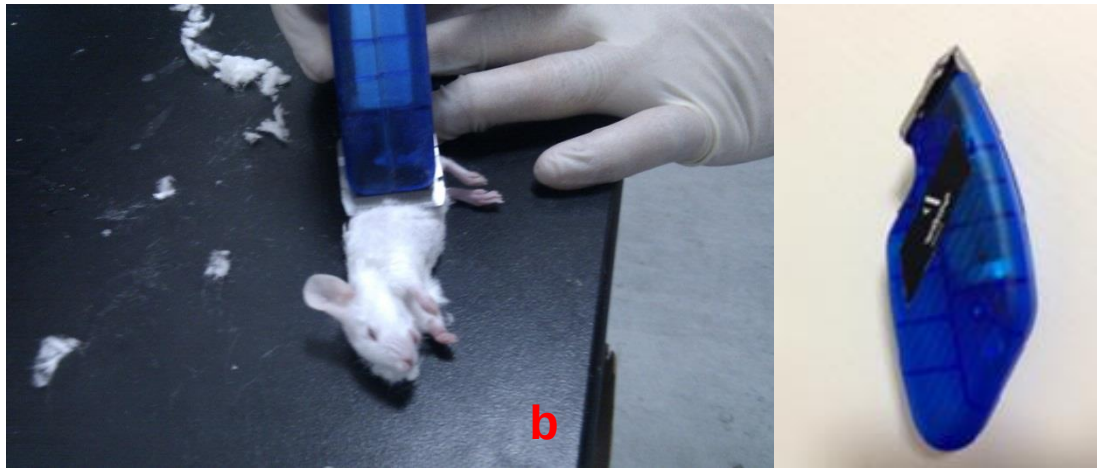
Depilation required

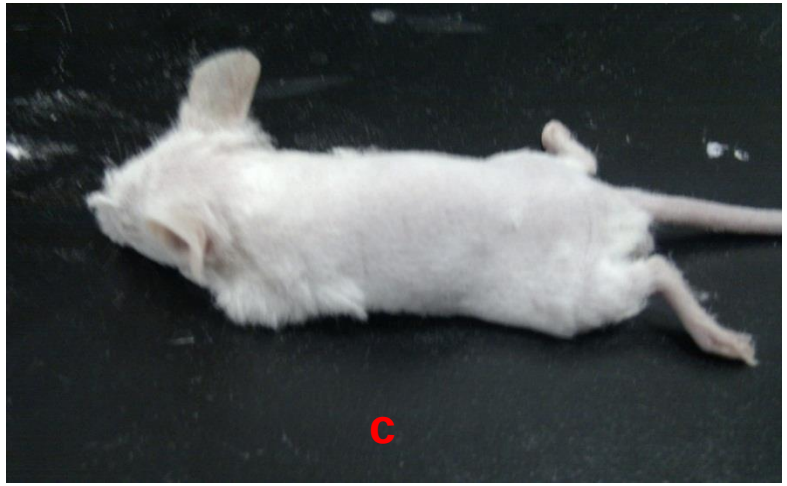
Normal, haired mouse strains
BALB/c, C57BL/6 etc . . .

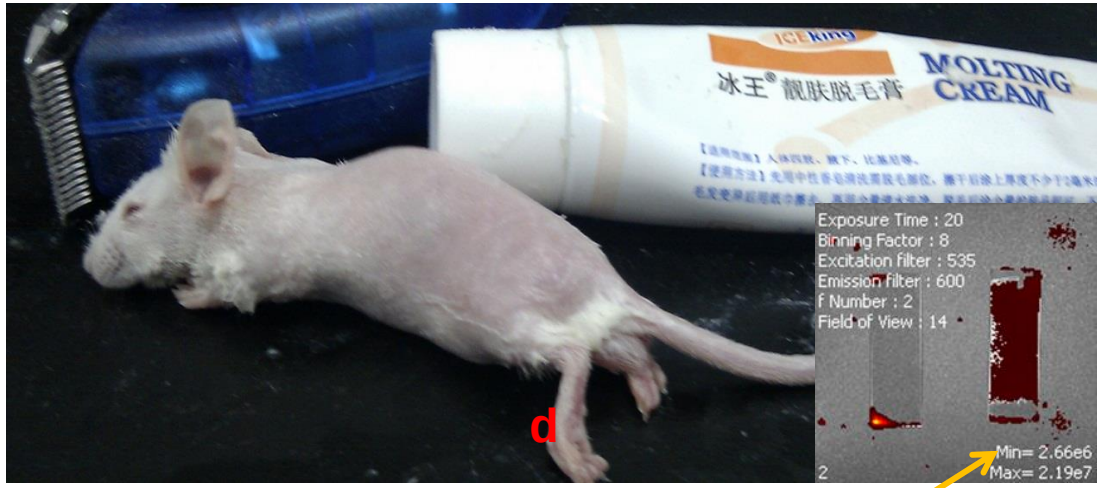
- **剃毛作用：降低毛发光吸收和光散射；去除来自毛发的背景荧光**
- **实验前一天剃毛**

剃（脱）毛方法



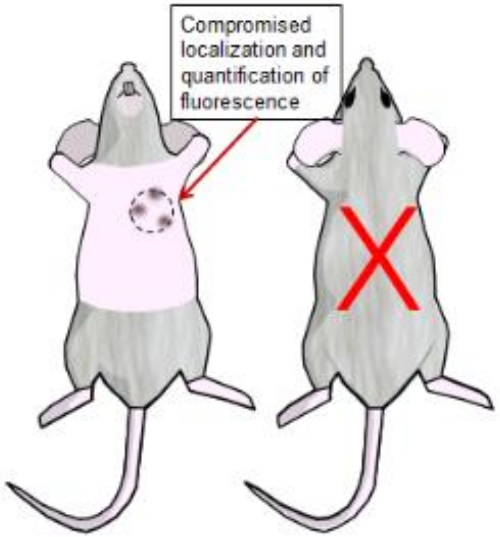


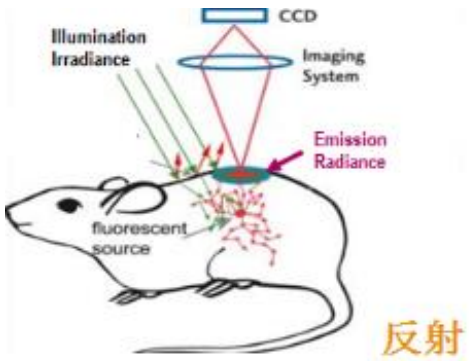




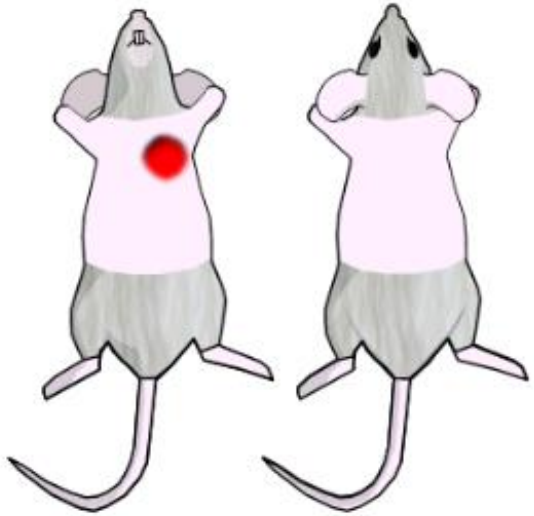
- a 剃毛前，体重16 g（约4 week）Balb/c 雄性小鼠；
- b 使用PerkinElmer剃毛器对小鼠进行剃毛；
- c 使用剃毛器后的效果，基于剃毛刀头的设计，有约1 mm的体表绒毛无法剃除；
- d 使用脱毛膏，可以将剩下的体表绒毛干净地去除，用完脱毛膏后用纱布蘸温水檫拭小鼠，避免脱毛膏的自发荧光干扰。

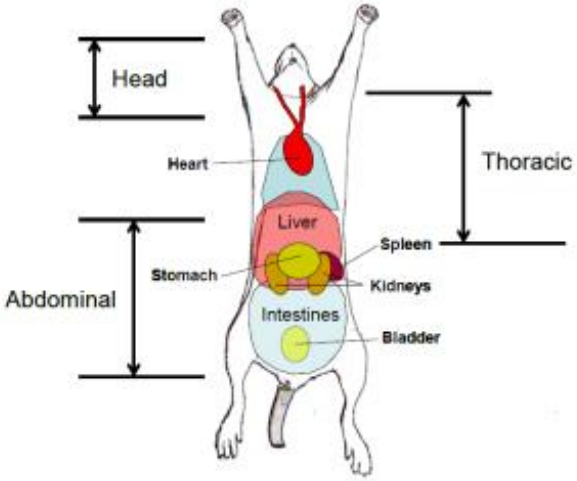
荧光成像小鼠剃毛——降低光吸收





- 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

荧光成像-光谱拆分

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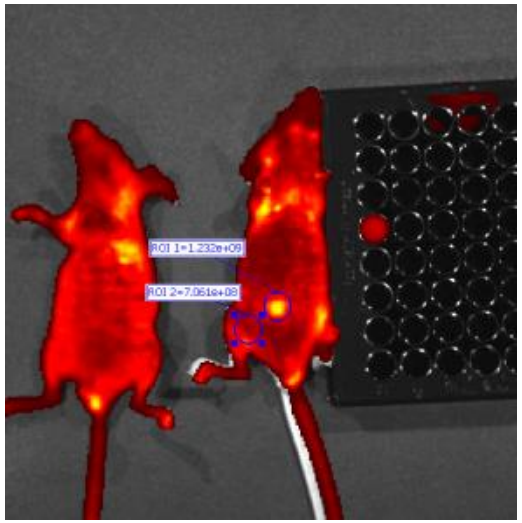
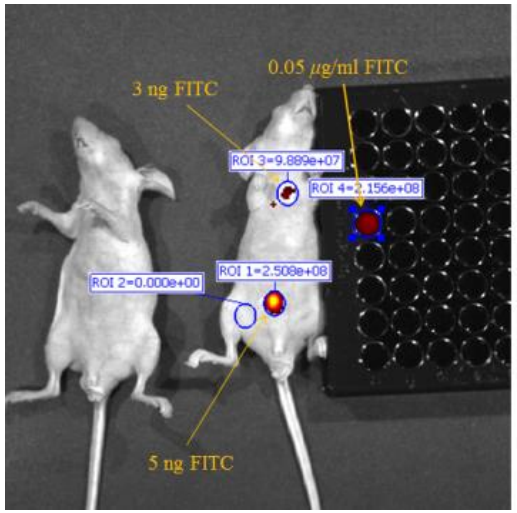
荧光成像的挑战——组织背景荧光

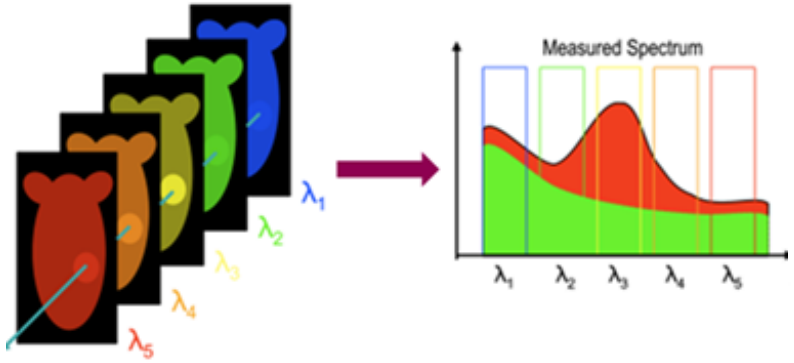


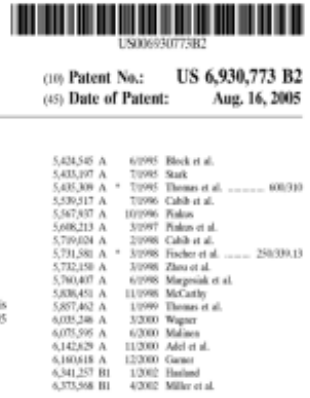
背景荧光



目标信号

光谱扫描硬件配置





US006930773B2

(12) **United States Patent**
Cronin et al.

(10) **Patent No.: US 6,930,773 B2**
(45) **Date of Patent: Aug. 16, 2005**

(54) **SPECTRAL IMAGING**

(75) Inventors: **Paul J. Cronin**, Charlestown, MA (US); **Peter J. Miller**, Newburyport, MA (US)

(73) Assignee: **Cambridge Research and Instrumentation, Inc.**, Woburn, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 273 days.

(21) Appl. No.: **10/226,592**

(22) Filed: **Aug. 23, 2002**

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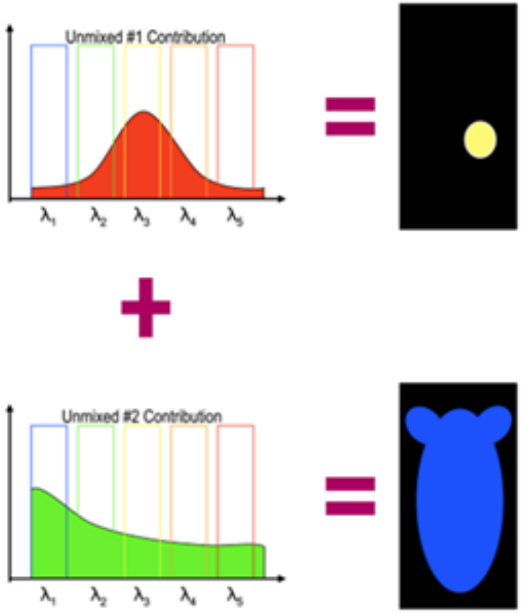
6,373,568	B1	4/2002	Miller et al.	

专利光谱拆分算法

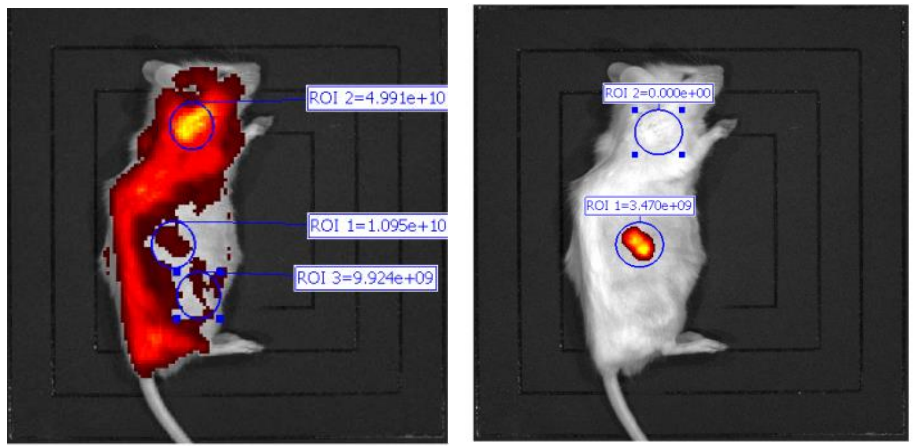


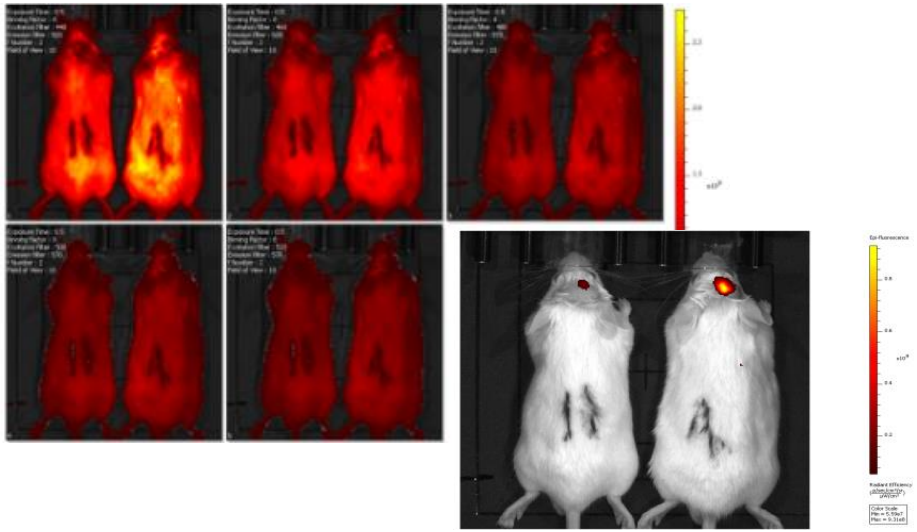
$$\begin{bmatrix} B_1 \\ B_2 \\ \vdots \\ B_{K-1} \\ B_K \end{bmatrix} = \begin{bmatrix} A_{1,1} & A_{1,2} & \cdots & A_{1,W-1} & A_{1,W} \\ A_{2,1} & A_{2,2} & & & \vdots \\ \vdots & & \iddots & & \vdots \\ A_{K-1,1} & & & \iddots & \vdots \\ A_{K,1} & \cdots & \cdots & \cdots & A_{K,W} \end{bmatrix} \cdot \begin{bmatrix} \exp\left[-\sum_{i=1}^{N} S_{1,i} c_i\right] \\ \vdots \\ \exp\left[-\sum_{i=1}^{N} S_{W,i} c_i\right] \end{bmatrix}$$

IVIS系统具有专利的荧光光谱分离技术，使用该技术已发表超过500篇文献

荧光成像-光谱拆分

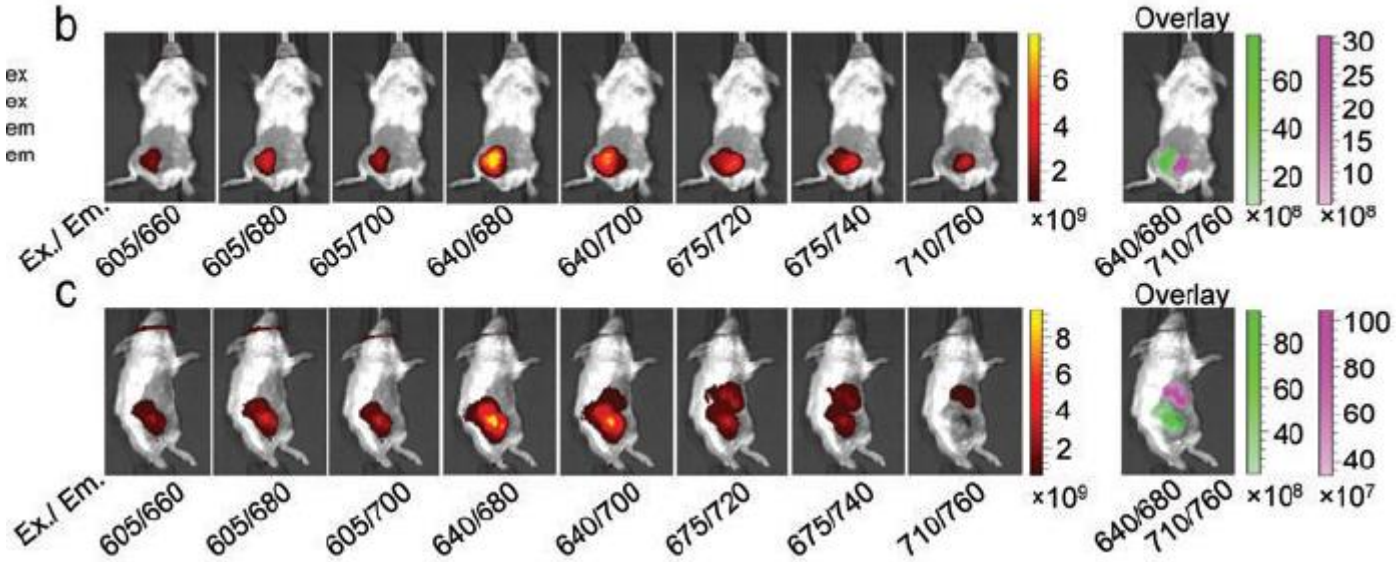
去除组织背景荧光



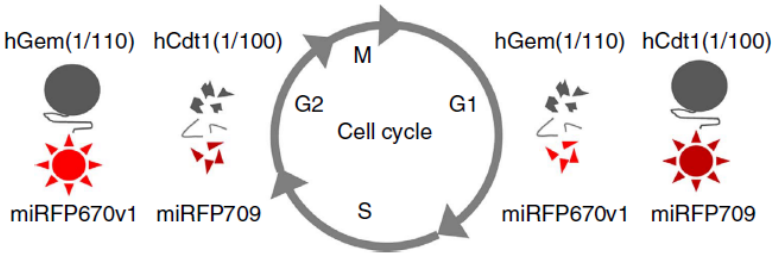


表达GFP的肿瘤细胞成像

多标记荧光的拆分

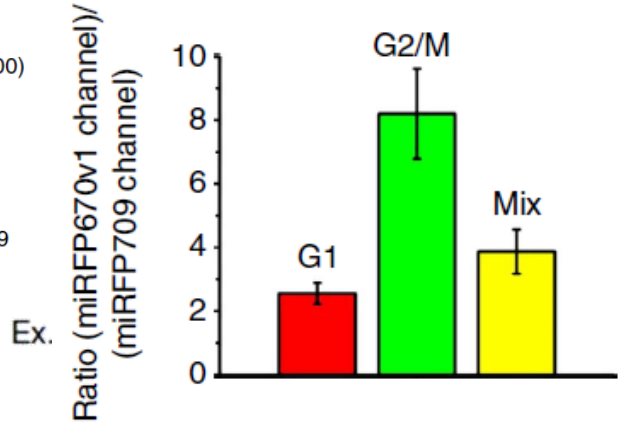


Shcherbakova and Verkhusha et al., ***Nat Methods***.2014



活体FUCCI监测细胞周期

NATURE COMMUNICATIONS, 2016 7:12405



生物发光-常用荧光素酶及分类

HUMAN HEAL

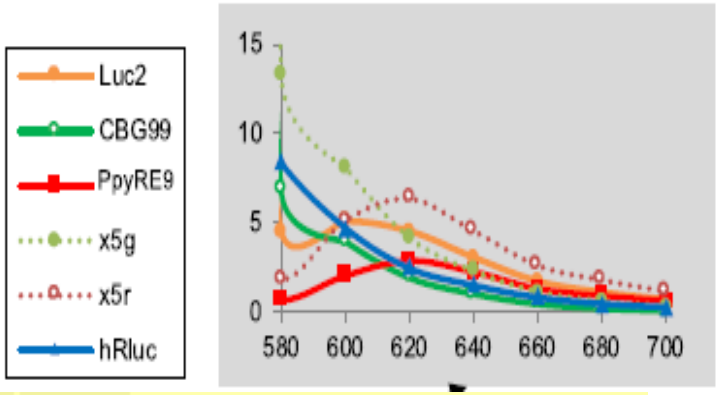
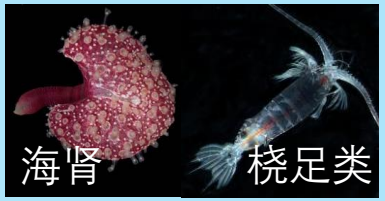


Table 1. Luciferase Reporters Commonly Used for *In Vivo* BLI and Their Specifications

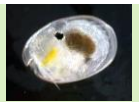
Bioluminescent reporter	Emission peak *in vivo*	Size (kDa)	Substrate	Cofactors	Codon optimized for mammalian expression	
Photinus pyralis; North American firefly luciferase	~600 nM	62	D-Luciferin	O_2, Mg^{++}, ATP	YES (Luc2)	[7,8]
Photinus pyralis red; North American firefly luciferase	618 nM	62	D-Luciferin	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



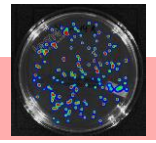
底物为腔肠素

海虾



海萤

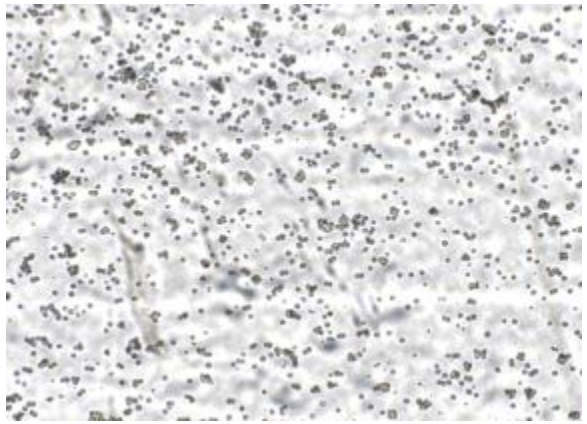
底物为海萤荧光素



改造的发光细菌

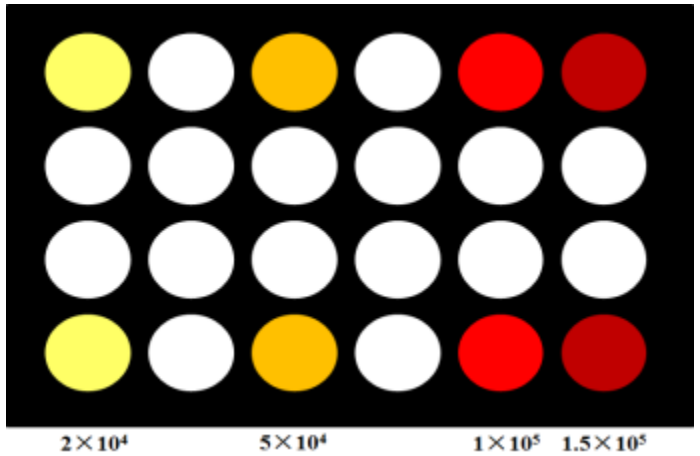
细胞生物发光效率测试

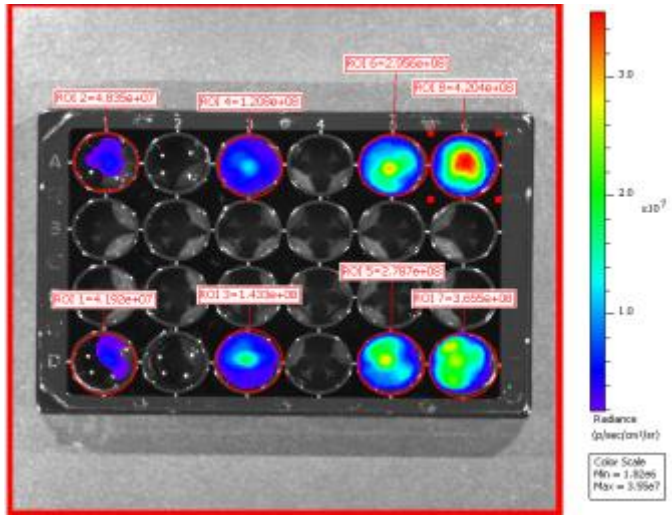
细胞计数



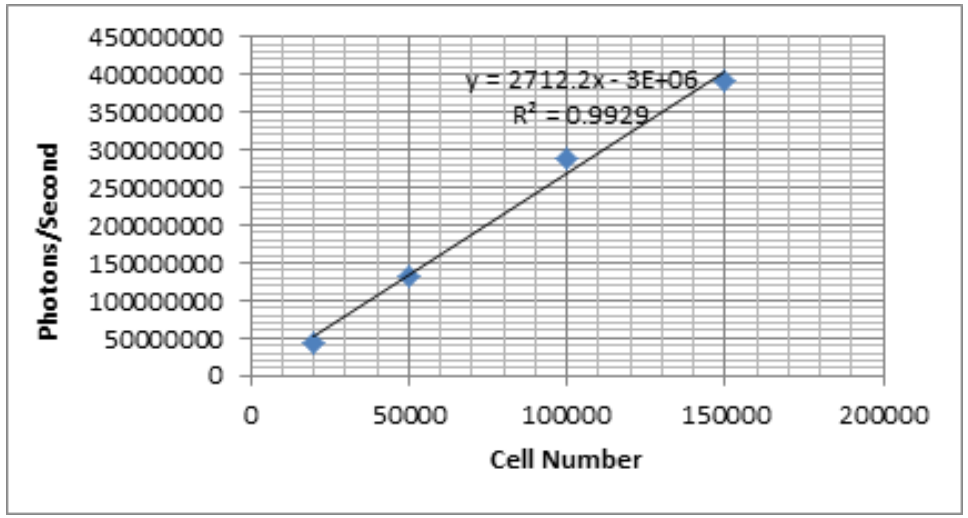
PerkinElmer #1450-605

梯度细胞种板





加入150 μg/ml D-luciferin底物溶液
3 min后使用IVIS系统成像

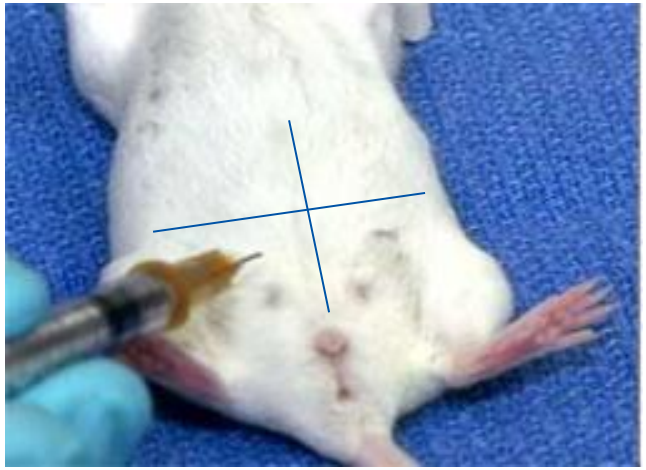


≥200

统计并计算Photons/sec/cell值

生物发光底物注射

腹腔注射



- 底物注射后10~20min进入成像平台期
- 2 h后信号消失

尾静脉注射

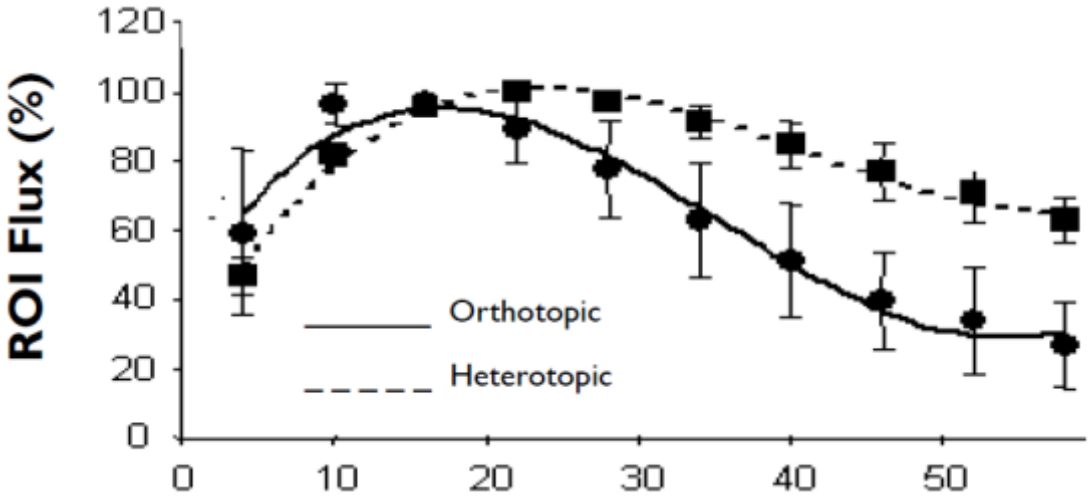
- 底物注射后约2min进入信号峰值
- 强度数倍于腹腔注射
- 约30min信号消失

底物剂量：150 mg D-Luciferin/kg 鼠体重

！！！对每个生物发光实验动物模型，首先需测一条生物发光动力学曲线，得出最适成像时间点

生物发光动力学曲线

1. 注射荧光素酶底物（建议在动物清醒时注射）；
2. 3 min后，麻醉动物（建议使用气体麻醉）；
3. 将麻醉后的动物放入成像仓中，在荧光素注射后约5min时刻拍摄第一张图片；
4. 之后每2 min拍一张，持续拍摄60 min，得到一条针对该实验模型的荧光素动力学曲线



原位和异位种植的Brain tumor的生物发光荧光素动力学曲线

- 依据上述曲线选取最佳成像时间点。
- 一般情况下，大多数实验模型的最佳成像时间点位于荧光素腹腔注射后10-20分钟。

其它成像前需注意

1. 不要使用标记笔在小鼠身上做标记；建议剪脚趾或在小鼠尾巴尖端做标记，并用黑纸板盖住；

2. 尽量选择异氟烷气体麻醉的方式，避免使用液体注射麻醉；

3.检测荧光弱信号，如果效果达不到预期，请尝试用黑纸板遮住其它有干扰的强信号后拍摄；

4.如果看肿瘤转移等微弱的生物发光信号，需要把较强的原位肿瘤信号遮住；

网络课堂

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CART 生物制药 纳米医药 IVIS

IVIS视角——[Nature]亚克隆合作通过修饰局部和全身的免疫微观环境驱动肿瘤转移

Original:张勇

IVIS视角 | 活体成像助力隐孢子虫感染可视化模型构建

Original:一只马鹿

IVIS视角 | 穿上 “细胞膜吉利服”的纳米载体在体内必将威力大增

Original:Teresa

IVIS视角 | 醛缩酶B介导的果糖代谢诱导了结肠癌肝转移过程中的代谢重组

Original:张勇

IVIS 视角 | 使用生物发光成像实时监测体内葡萄糖摄取

Original:Sherry

IVIS视角 | 更灵敏的炎症反应检测——光动力诊疗双功能探针开发

转化医学系列 | 液闪检测在肿瘤治疗的不同理念中的应用

观看回放

转化医学系列 | 仿生纳米材料在脑胶质瘤治疗中的应用探索

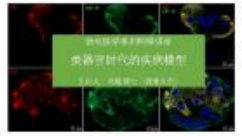
观看回放

转化医学系列 | 高内涵筛选平台助力药物研发实例分析

观看回放

转化医学系列 | BRD4和ALKBH5的活性测定方法建立及其抑制剂的发现

观看回放



转化医学系列 | 类器官时代的疾病模型

观看回放

转化医学系列 | 小动物多模态成像技术在抗癌药物研究中的应用

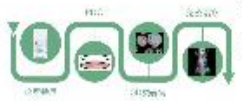
观看回放

转化医学系列 | 多能干细胞神经分化技术在药物发现及细胞替代治疗上的应用

观看回放

3D仿生生理和病理微组织阵列技术助力药物研发和临床治疗

观看回放



精准医疗系列 | 从拉罗替尼获批到新一代TRK抑制剂药物研发

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