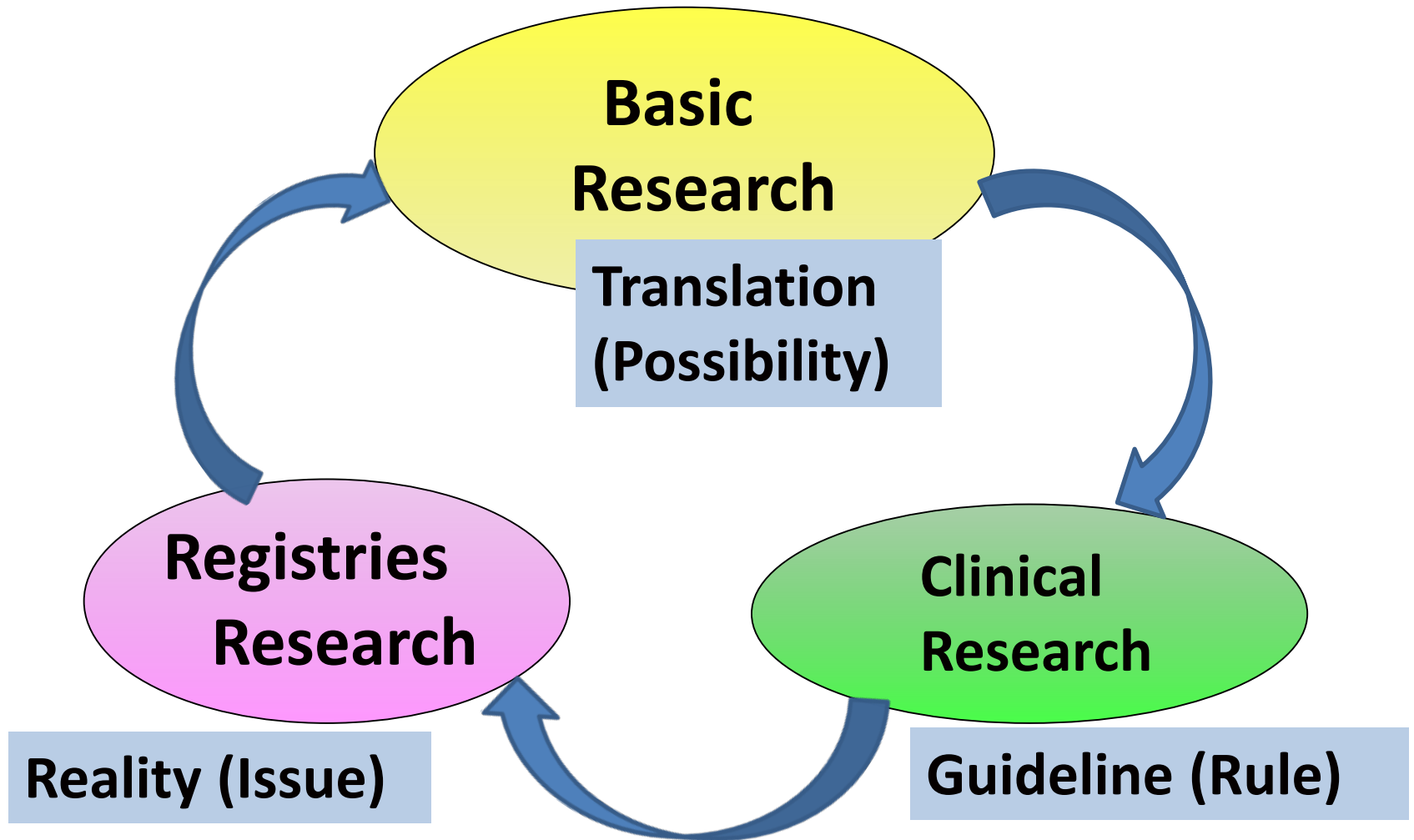


# Why doctors are in need of pushing forward researches?



**Eiji Kobayashi, MD, PhD**  
**Department of Organ Fabrication,**  
**Keio University School of Medicine, Japan**

# The reason why doctors need to continue researches

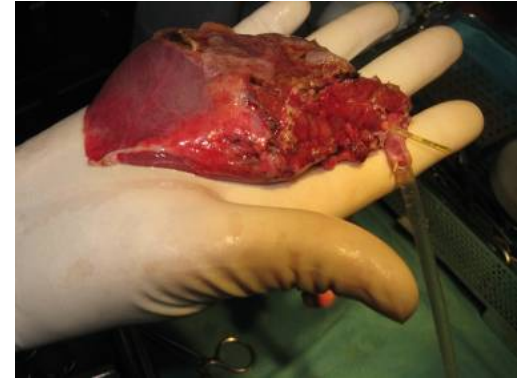
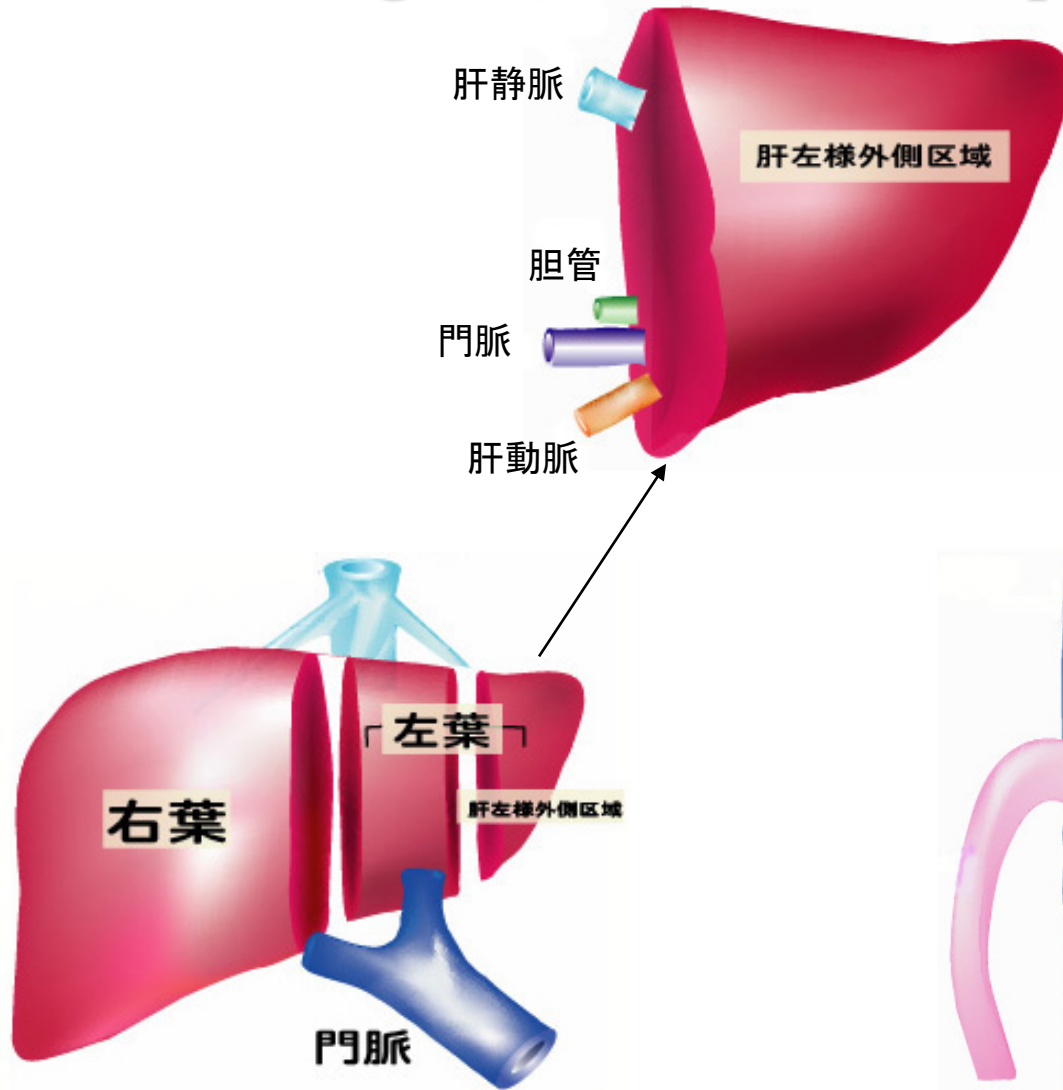


A powerful tool for **Translational Research**

*Microsurgery*

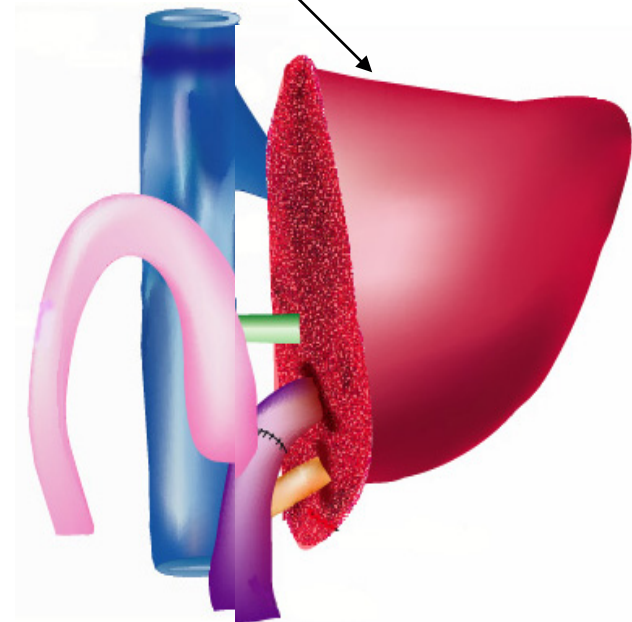


# Why microsurgery is in need in Living Donor Liver Transplantation in Children



S2 Graft (93g)

(*Am J Transpl*, 2010)







# Prof Ti-Sheng Chang, Fathre of Microsurgery in China



小林英司教授指正:

张涤生 敬  
上海交通大学附属  
东方医院。  
2010.4.21

2010/04/22 16:08  
Ti-Sheng Chang

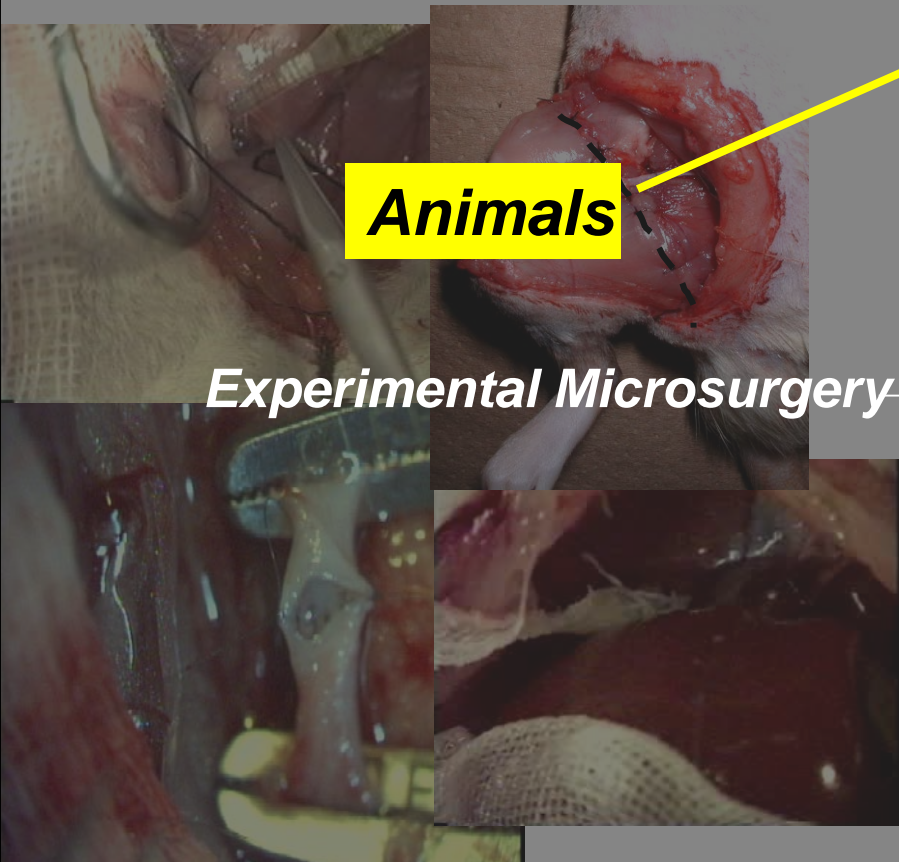




# *Prof Sun Lee, Father of International Society for Experimental Microsurgery (ISEM)*

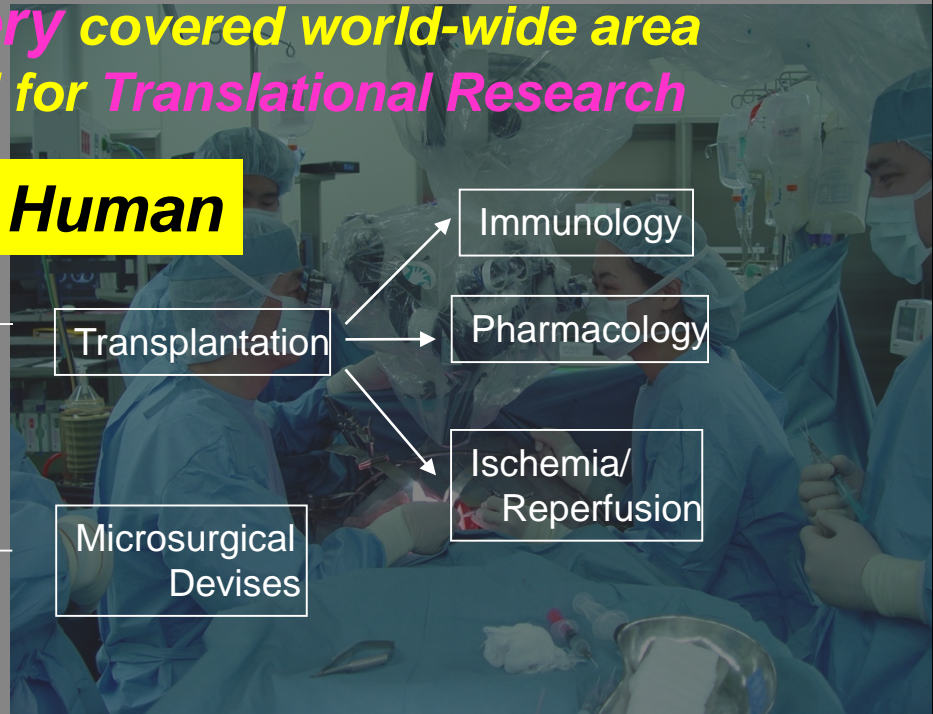


*Experimental Microsurgery covered world-wide area as a powerful tool for Translational Research*



**Animals**

**Human**



Transplantation

Immunology

Pharmacology

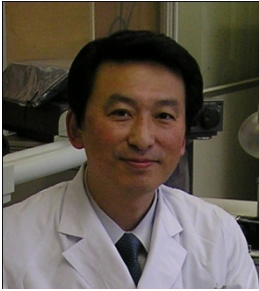
Ischemia/  
Reperfusion

Microsurgical  
Devises

Tissue/Organ Engineering

Minimum Invasive  
Surgery

*Experimental Microsurgery*



## Professor Eiji Kobayashi

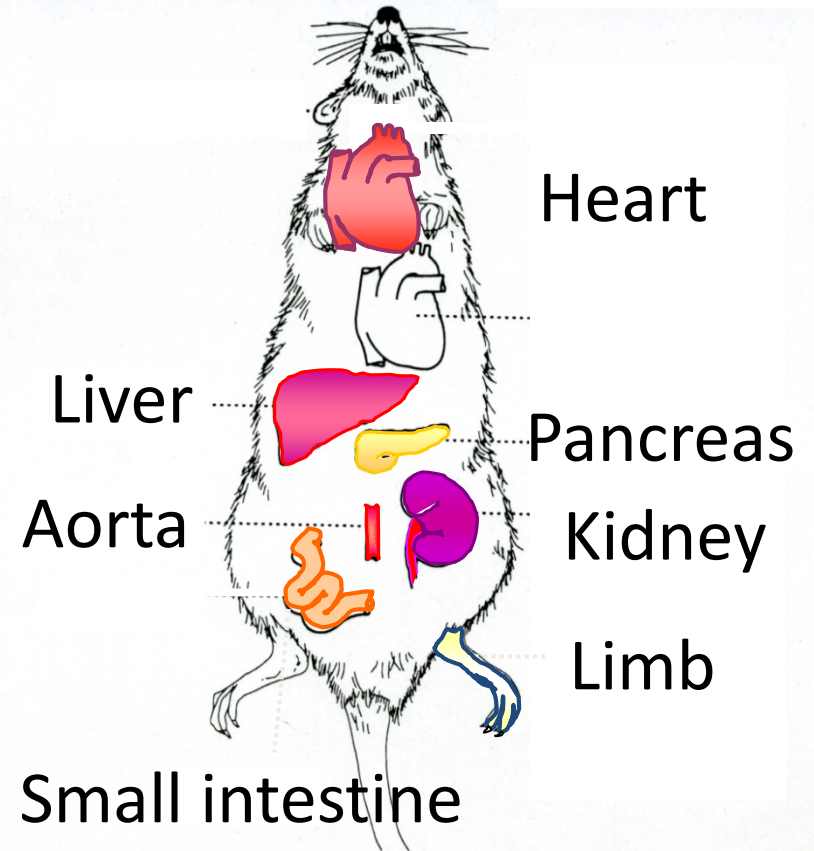
1982 Graduation from Jichi Med Sch  
2001 Professor, CMM and Dep Surgery  
2003 Director, CEM, Jichi Medical Univ  
2009 Chief Scientific Adviser, Otsuka  
2014 Professor, Keio University, Sch of Med



The 10<sup>th</sup> President; International Society for  
Experimental Microsurgery (ISEM)



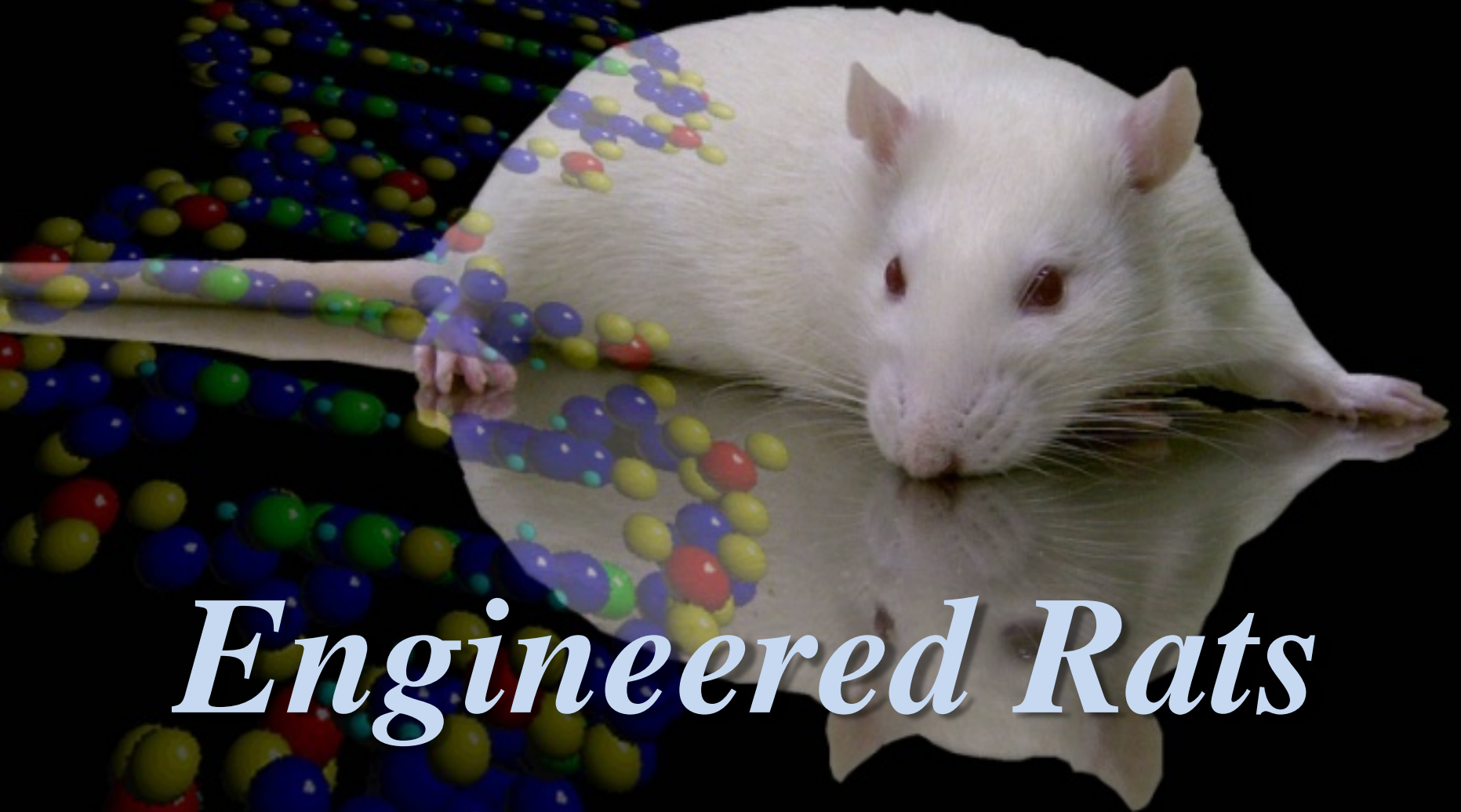
*Almost all organs can  
be transplanted in rats*



*In his Labo, you can muster excellent technique.*



A powerful tool for **Translational Research**



*Engineered Rats*

# GFP Animals in JMU



ウェーデンの王立科学アカデミー  
物理学研究所・元上席研究員とな  
た。下村さんは、オワンクラゲ  
(GFP)を分離し、その構造を  
子にくっつけて追跡する便利

ラット



マウス



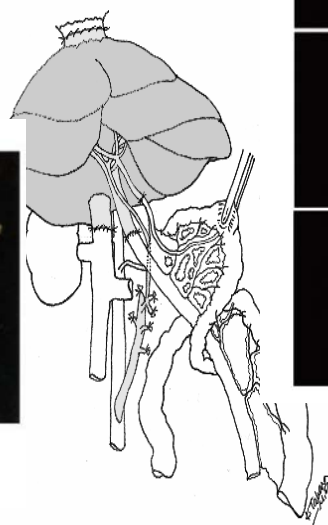
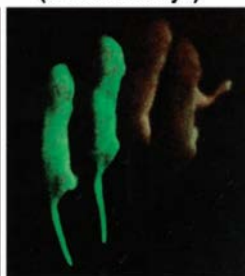
(2007)

賞を米ウツ  
氏の計3人  
で、緑色蛍  
の研究で、  
に与われて

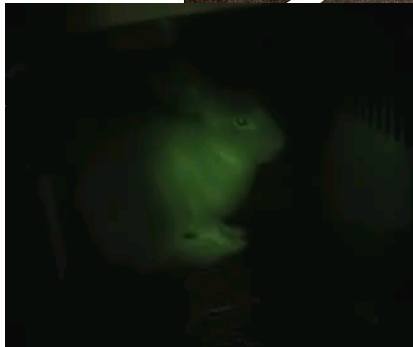
(2000) ニューセッツ州の自宅  
ーベル化学賞受賞の連絡を受けた後  
話で喜びを語る下村脩さん=A P

可起ホ (visible rays)

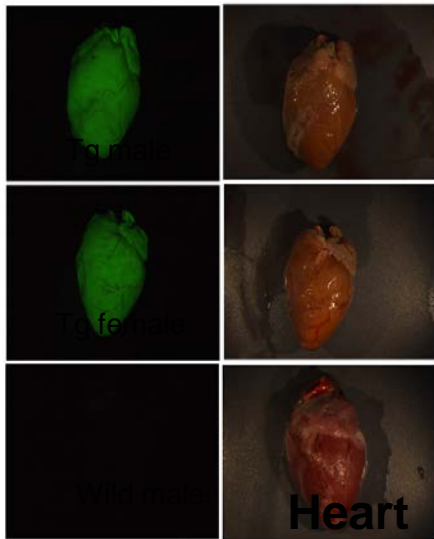
中 (ultraviolet rays)



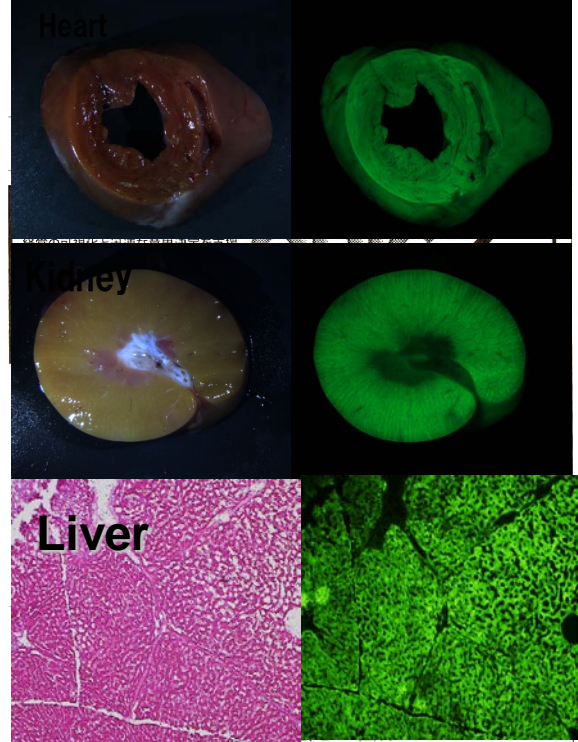
ウサギ



(2006)



千葉 (2007)  
東京



かの県警に広がることには否定的。反対派もおり  
議論を呼びそうだ。 **35面**

検討する。

GFP (+) (+) (-) (-)  
Fiy (+) (+) (-) (-)

ラット  
マウス

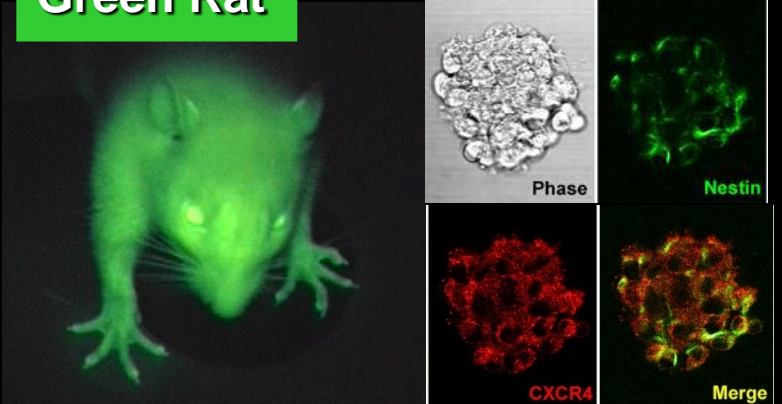
ーだ  
見して  
心用な  
るのだ  
いから  
になつ  
んばく

氏



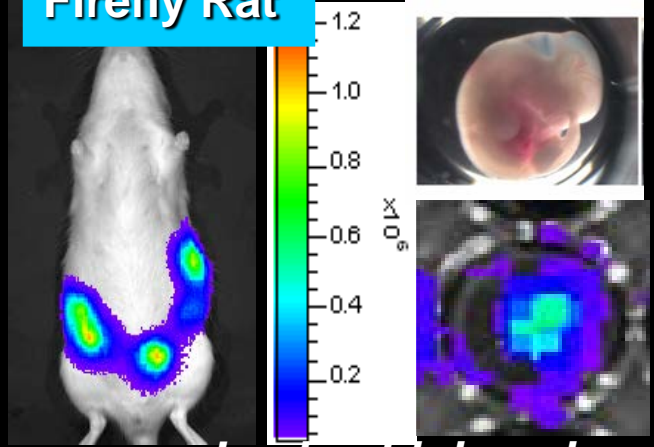
# A Powerful Tool for Translational Research - Engineered Rats established in JMU-

Green Rat



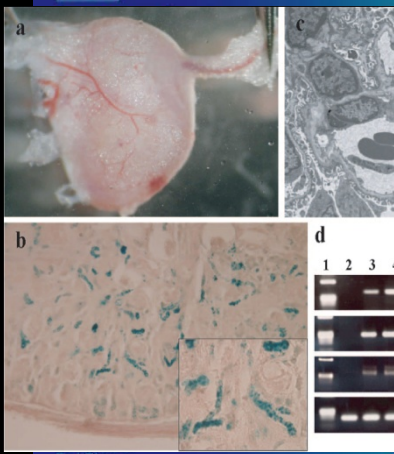
Neuroscience

Firefly Rat



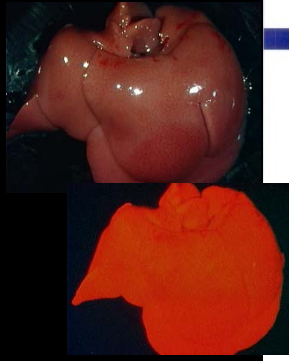
In vivo bioimaging

Blue Rat



Development

Red Rat



Gene Therapy

ISSN 0969-211X  
Volume 120, Number 1, April 1, 2001

**B  
B  
R  
C**

Biochemical and  
Biophysical  
Research  
Communications

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www.transplantjournal.com January 27, 2005  
Volume 79 - Number 2

**Transplantation**  
THE OFFICIAL JOURNAL OF THE TRANSPLANTATION SOCIETY

Gene silencing in transplanted tissues by siRNA  
(pp. 240-243)



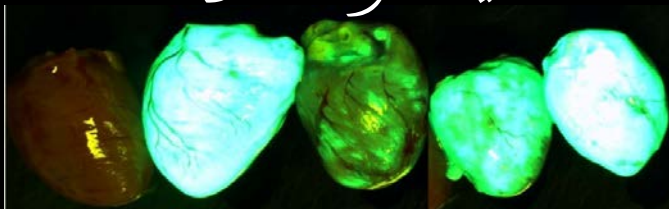
# Transgenic Technology in Rats

1. A few success rate
2. Random Integration by chance!!

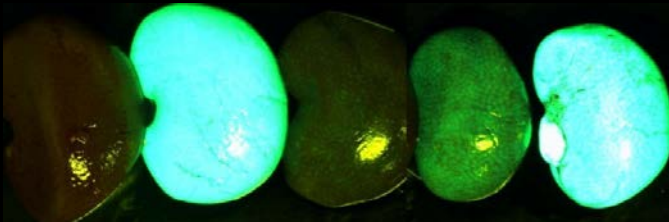
## Green Rats

Wild rat  
Wister(N)  
Wister(O)  
Fisher344  
Lewis

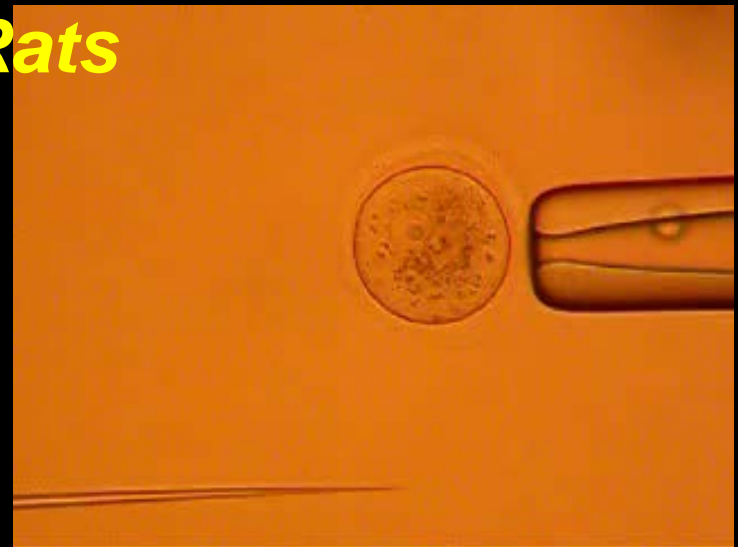
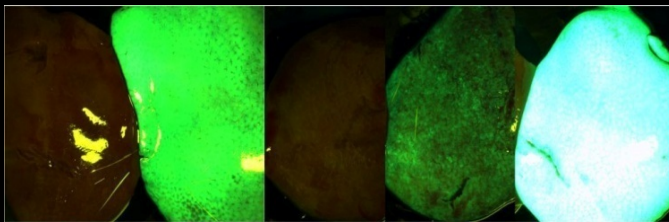
Heart



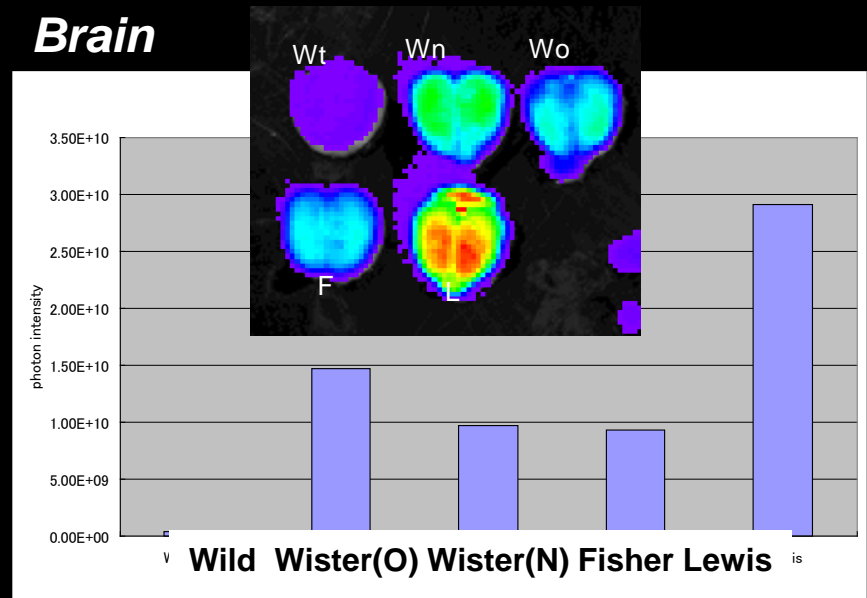
Kidney



Liver



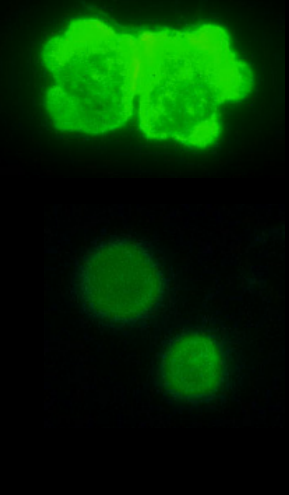
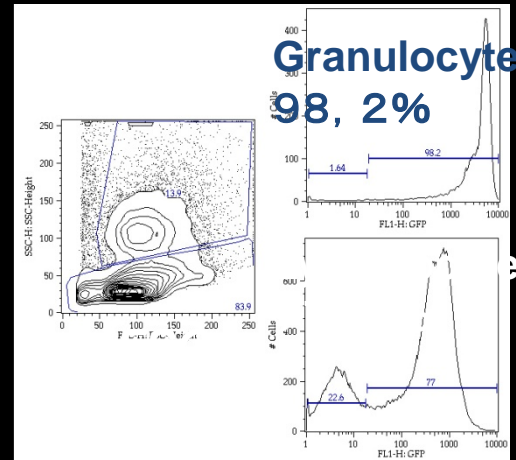
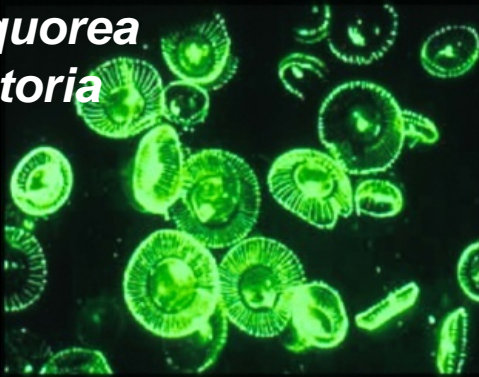
Brain



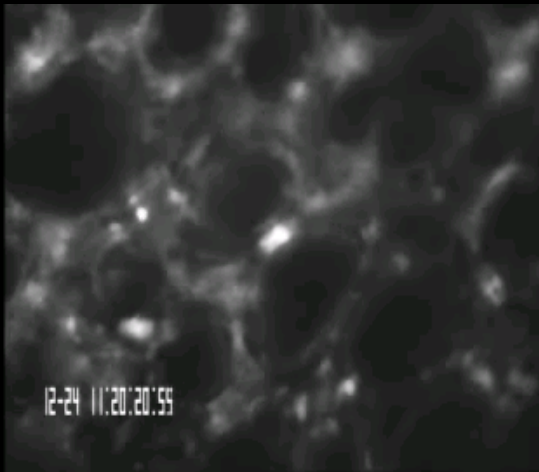
(Hakamata Y & Kobayashi E. 2006)

# Benefits in bio-imaging system using 'fluorescence'

*Aequorea victoria*

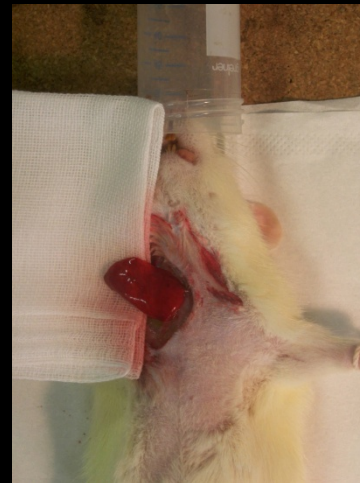


(Sakuma Y, et al. *Transplant Immunol* 2004)



Lung; 25 min after LPS injection

(Sato A, et al. *Anesth Analg* 2005)



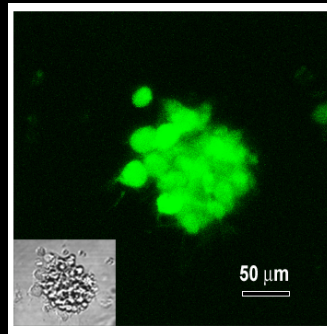
Transplanted lung immediately after re-perfusion

(Enomoto A, et al. *Microsurgery* 2007)

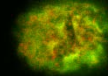


# Differentiation from the fetal tissue

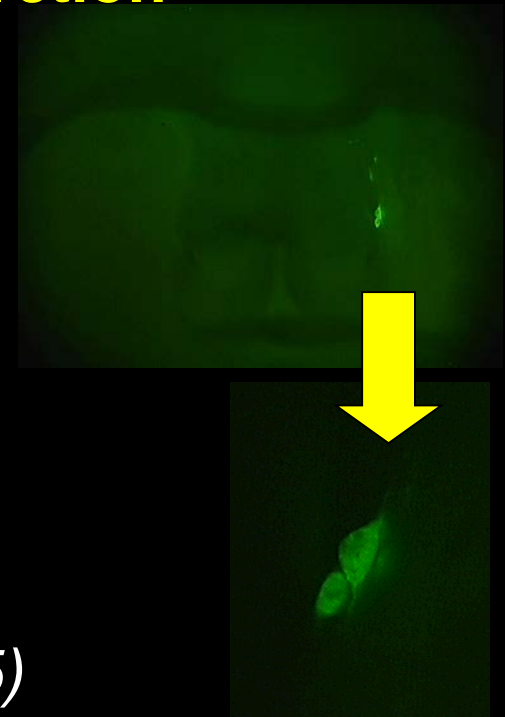
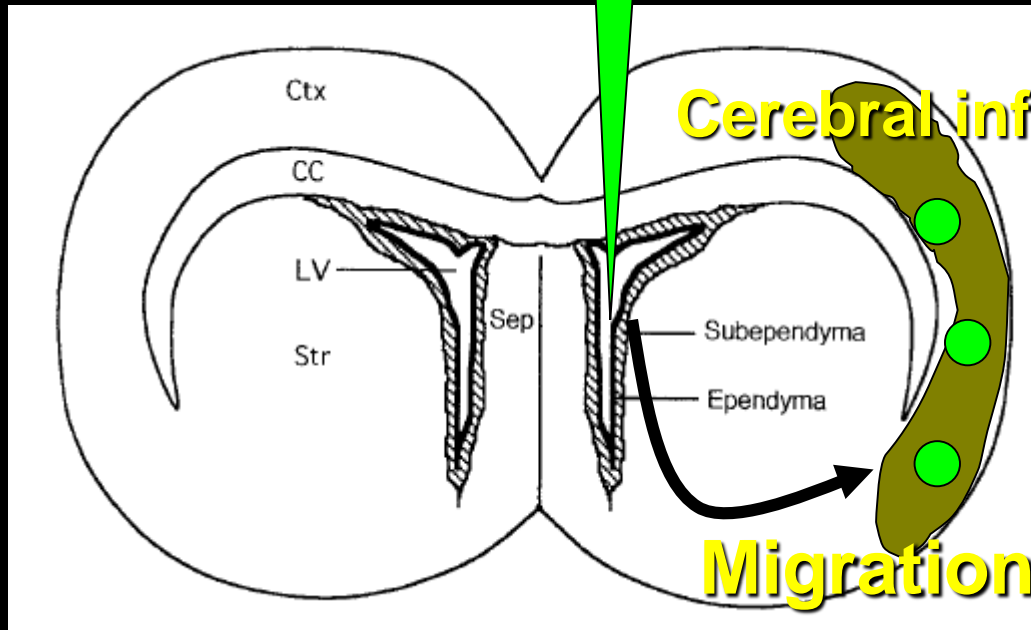
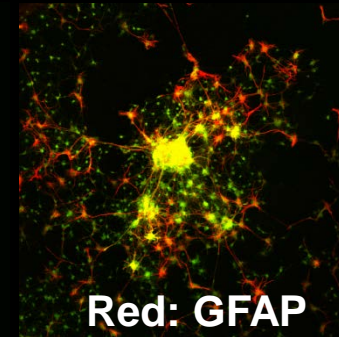
Neurosphere



Red: Nestin

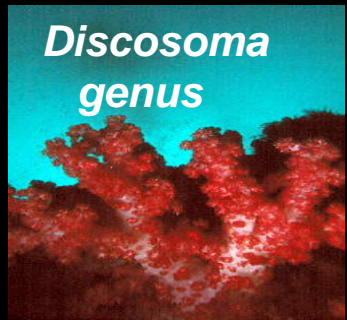
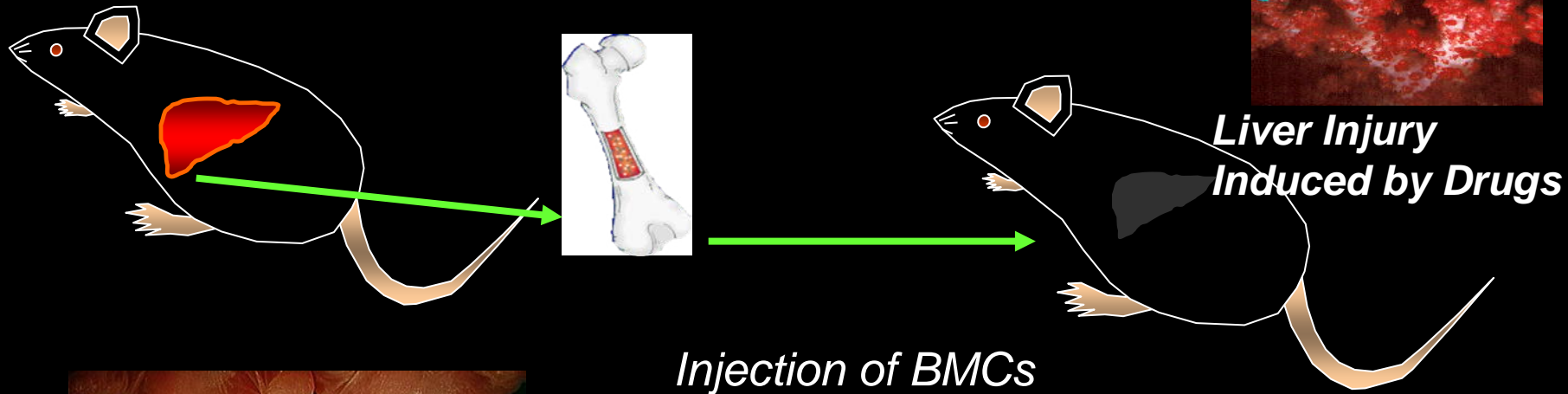


Red: GFAP



(Inoue H, et al. *BBRC* 329:288,2005)

# Application for Hepatology



Injection of BMCs

You can see the transdifferentiated cells



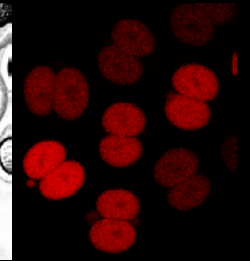
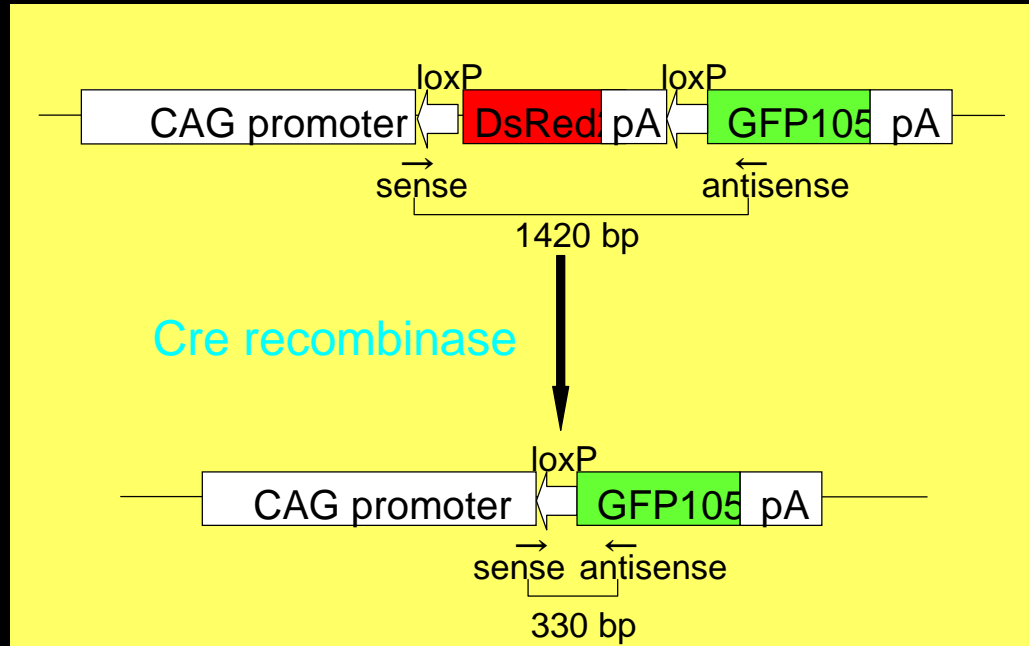
Liver Sample x400

(Sato Y, et al. BBRC 2005)

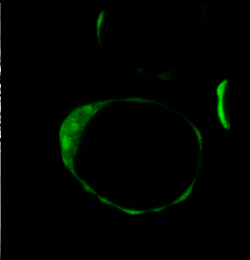
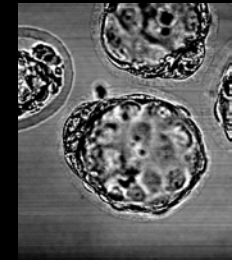




# Double Reporter (Red to Green) Tg; Cre/LoxP recombination system



After recombination

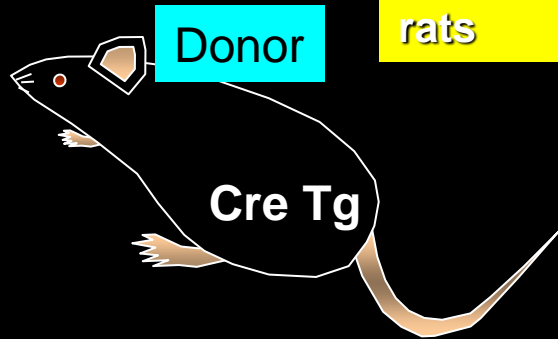


- CAG promoter driven DsRed2/GFP double-reporter Tg rats
- Cre recombinase expressing Tg rats

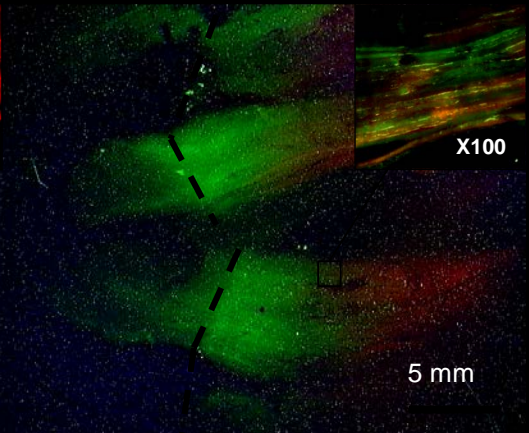
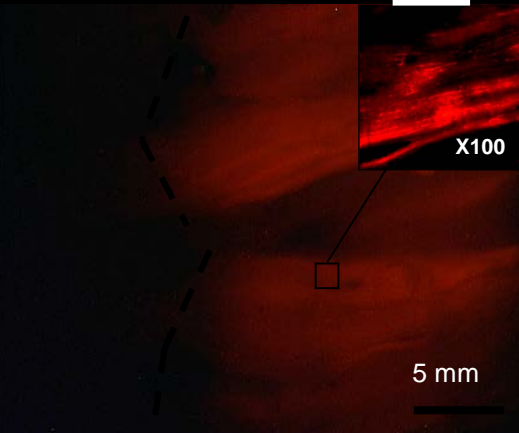
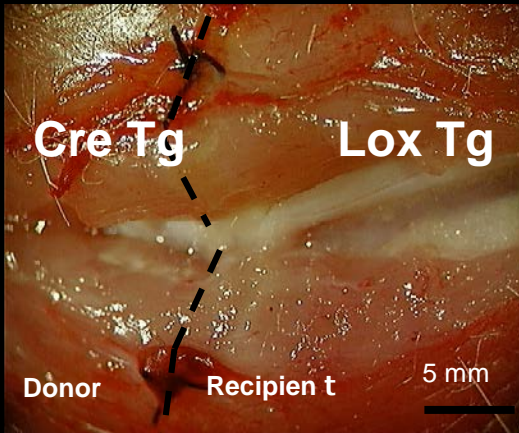
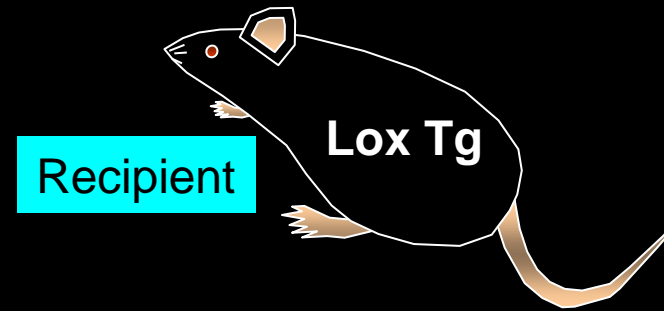
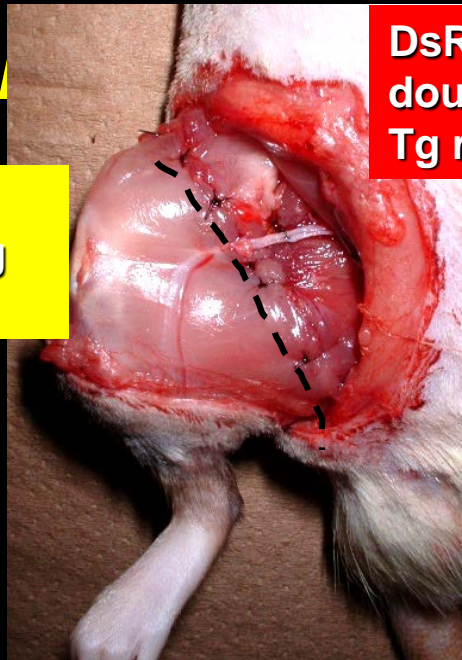
( Sato Y, et al.BBRC 319:1197,2003)

# Identification of muscle fusion events in limb

transplant



Limb graft from Cre Tg rats



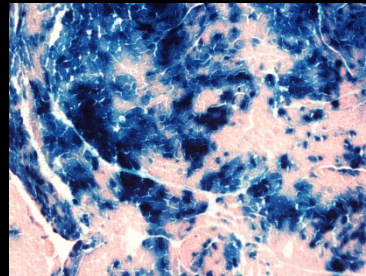
# B u l e R a t s

Enbrio 21. 5 days



*Skeletal Muscle*

*Heart*



( Takahashi M, et al. BBRC 2003)

ISSN 0006-291X  
Volume 329, Number 1, April 1, 2005

**BBRC**

*Biochemical and  
Biophysical  
Research  
Communications*

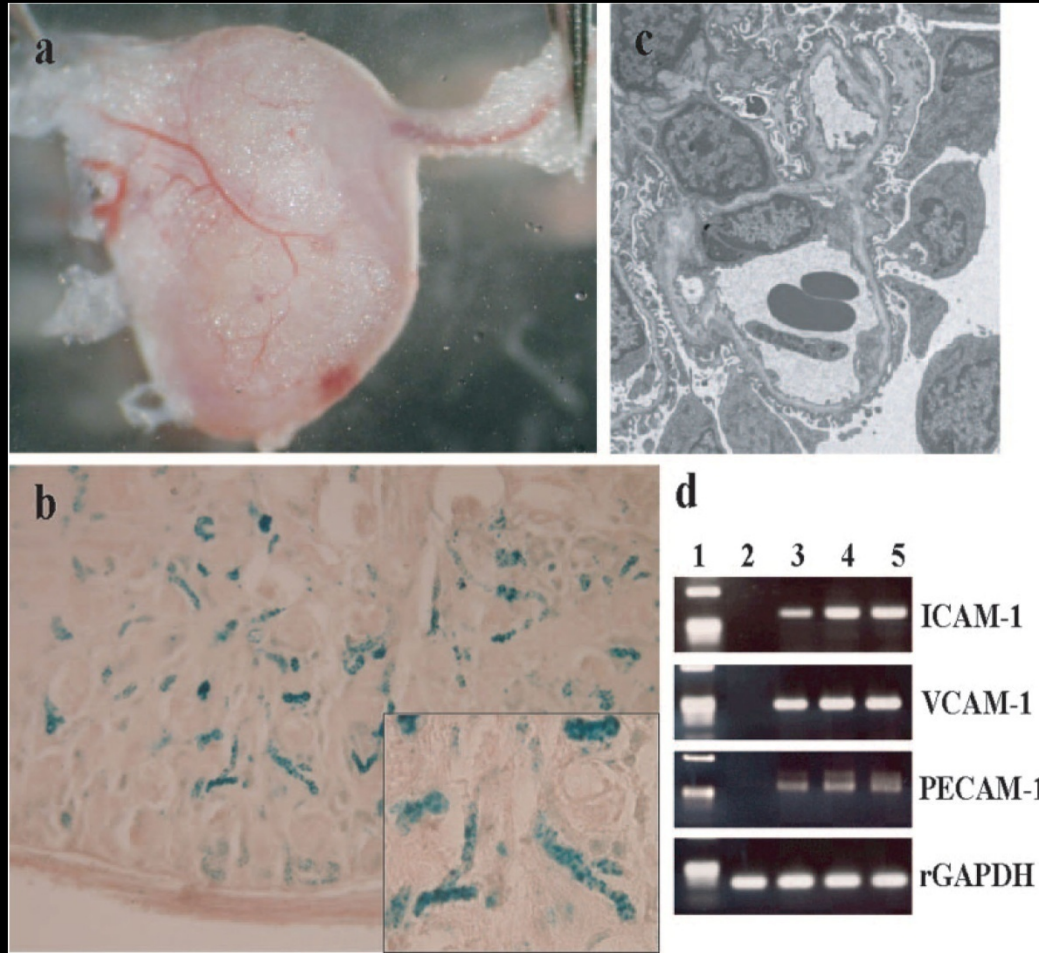
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Mao-Ming Poo  
Jacques Pouyssegur  
William S. Reznikoff  
Kiyoshi Takatsu  
Naoyuki Taniguchi

( Inoue, Ohsawa et al. BBRC 2005)



# Transplantation of neo-kidney into the LacZ Tg rat host



(a) Stereoscopic microscopy

(b) X-gal assay

(c) Electron microscopy

(d) RT-PCR using FACS-gal assay

(Yokoo T, et al. *J Am Soc Nephrol* 17;1026,2006)

# Proceeding of International Collaboration will encourage us very much !



**Bonn Univ**

**Germany**  
EK Geissler (Regensburg)  
G Nikkhah (Freiburg)  
R Tolba (Bonn)



**Regensburg Univ**



**Freiburg Univ**



**Toronto Univ**

**Canada**  
A Keating (Toronto Univ)



**Missouri Univ**

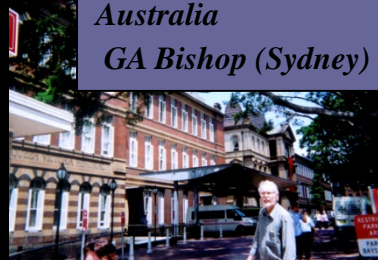
**U.S.A**  
John Critser (Missouri Univ)  
SS Thorgeirsson (NCI)  
P Leone (RWJ Med Sch)



**Thailand**  
A Sereemaspun  
Singapore  
DW Hutmacher  
S Cool (Bioporis)



**Australia**  
GA Bishop (Sydney)

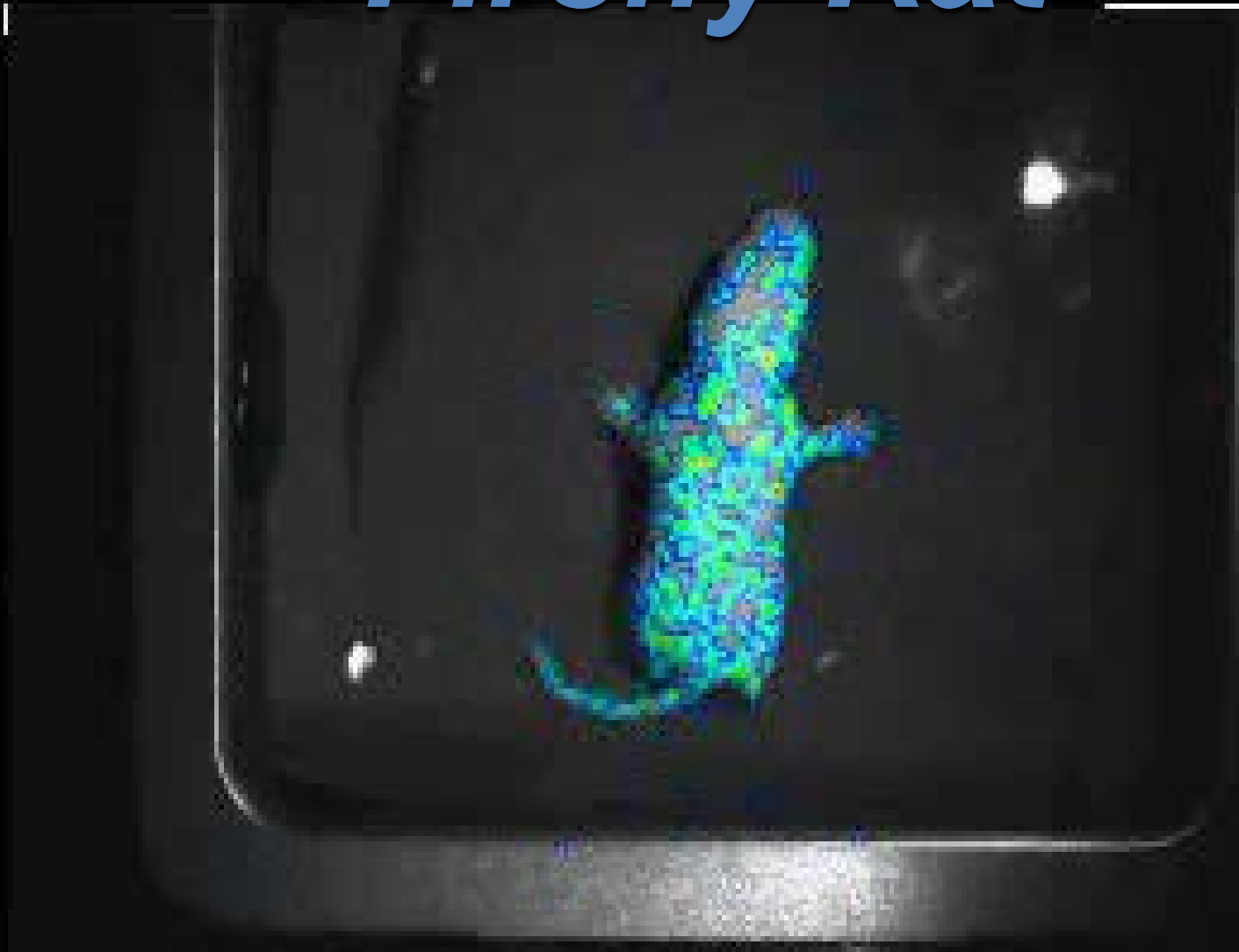


**NCI**



**RWJ Med Sch**

# *Firefly Rat*





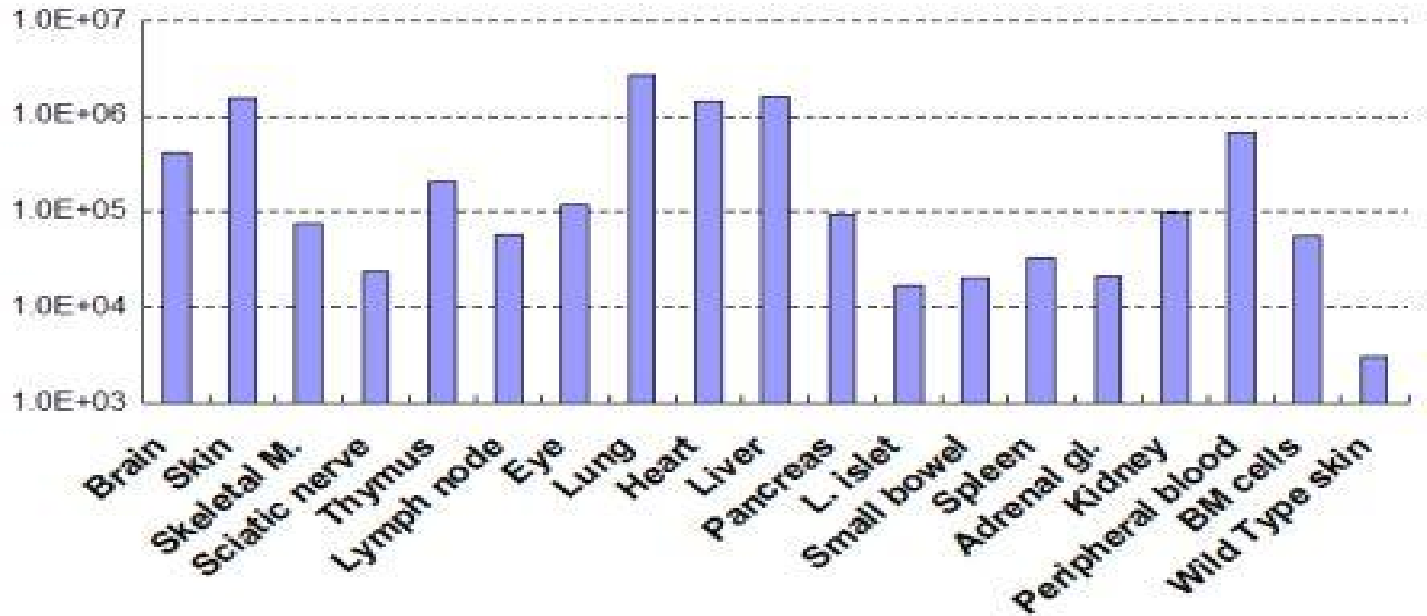
# 上海交通大学 医学部 大学院特别講演

## Stem Cell Research Using Bioimaging Rat and Pig

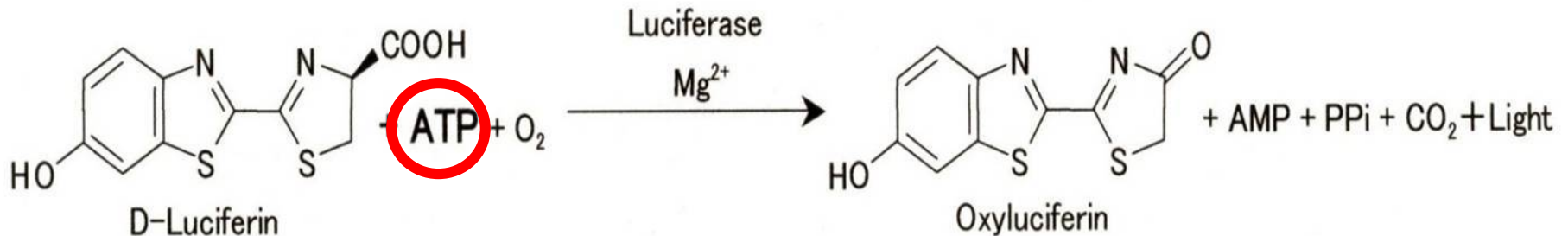


2010/04/21 10:31

# Luciferase Transgenic Ratの発光



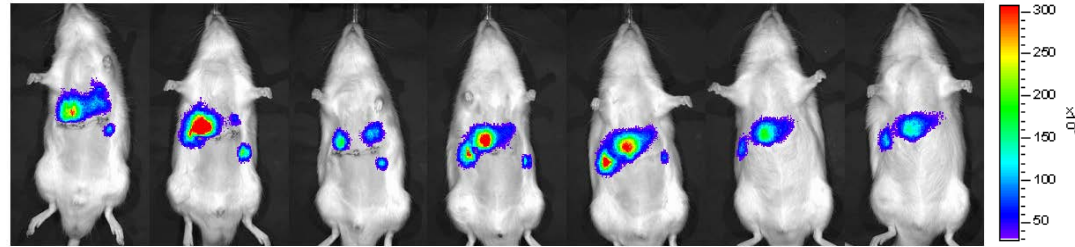
(Hakamata Y, et al. Transplantation 2006)



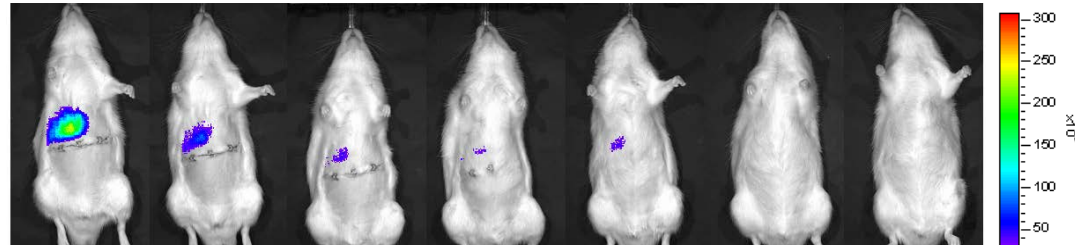


# Case the transplanted hepatocytes

*Hepatectomized*



*Normal*



POD

1

3

7

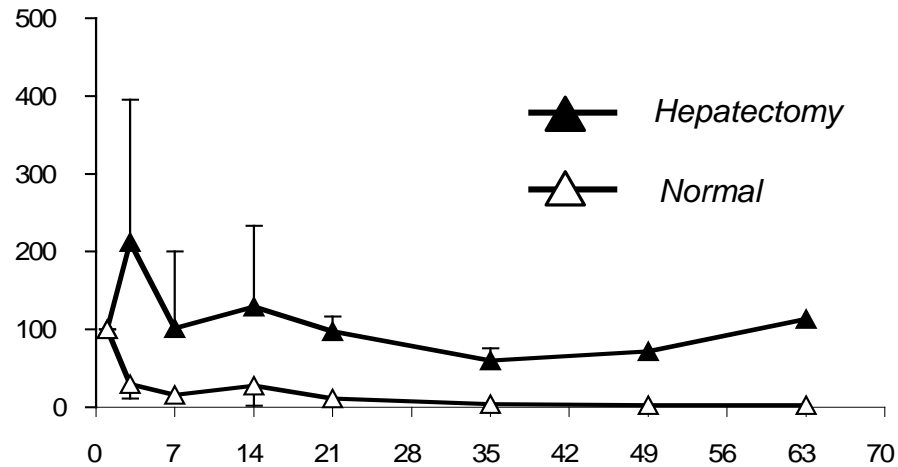
14

21

35

63

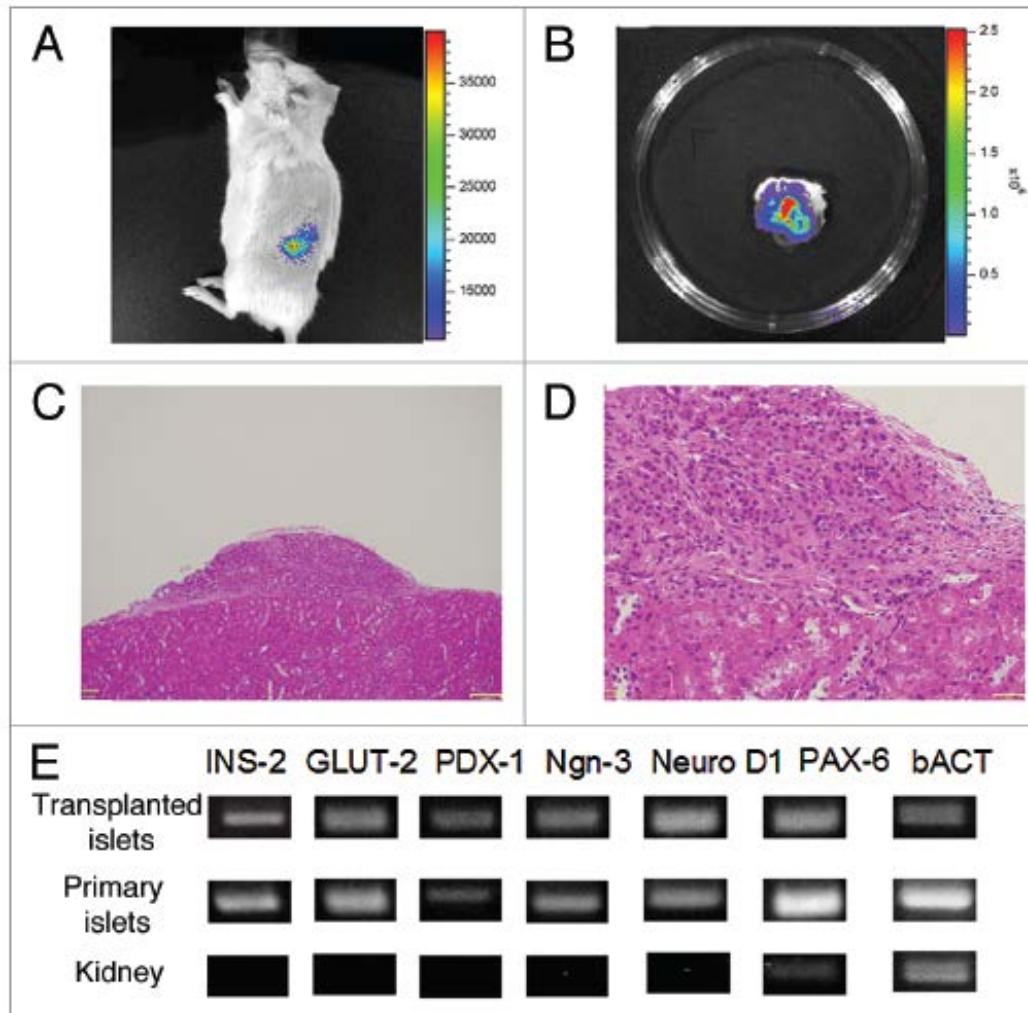
Relative expression index



Days after hepatocytes transplantation

(Hakamata Y, et al Transplantation 2006)

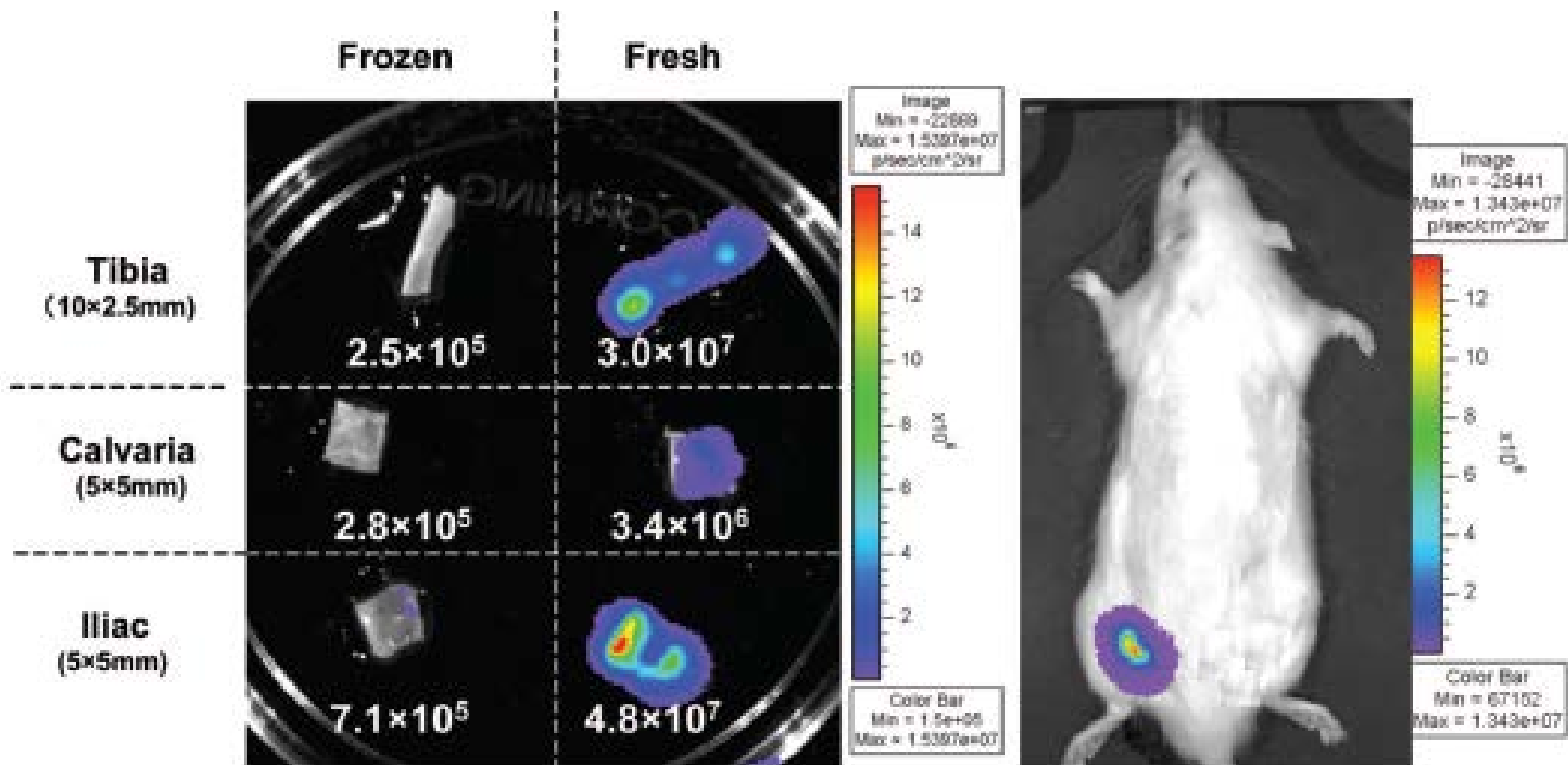
# Luminescence Technology in preservation and transplantation for rat islet



( K Negishi et al. *Islets* 3(3):111-117 2011)



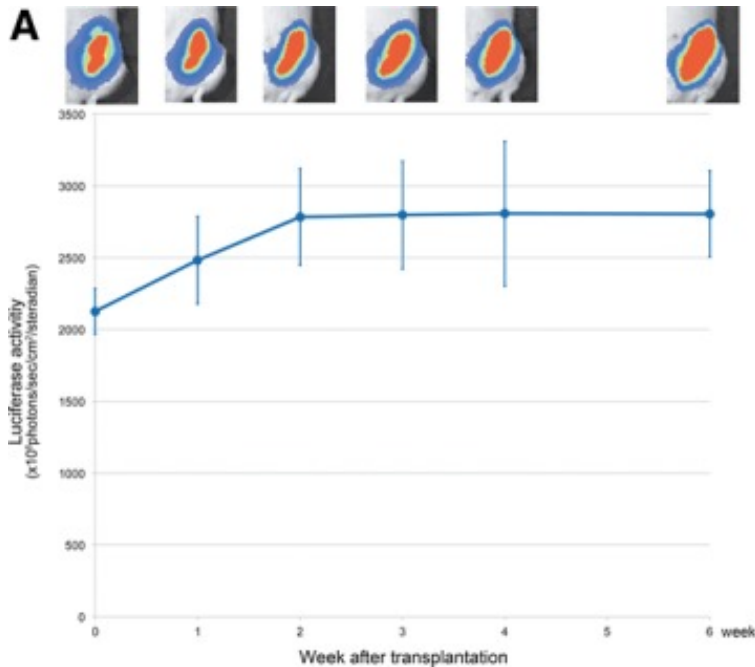
# Luminescence Imaging of Regenerating Free Bone Graft in Rats



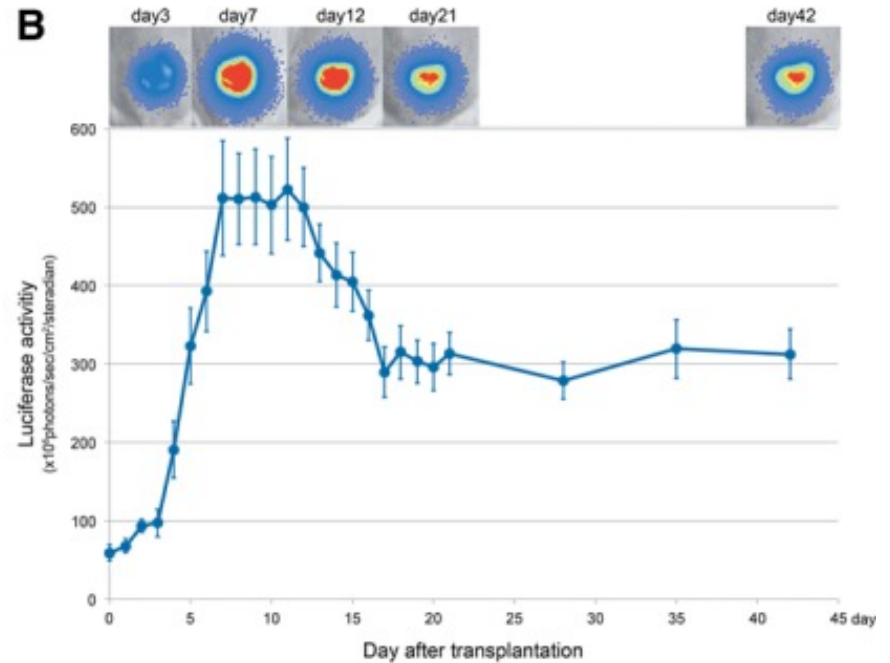
(A Yamaguchi et al. *Plast Reconstr Surg* 127(1):78-87 2011)

# The Fate of Nonvascularized Fat Grafts: Histological and Bioluminescent Study

## Vascularized Graft

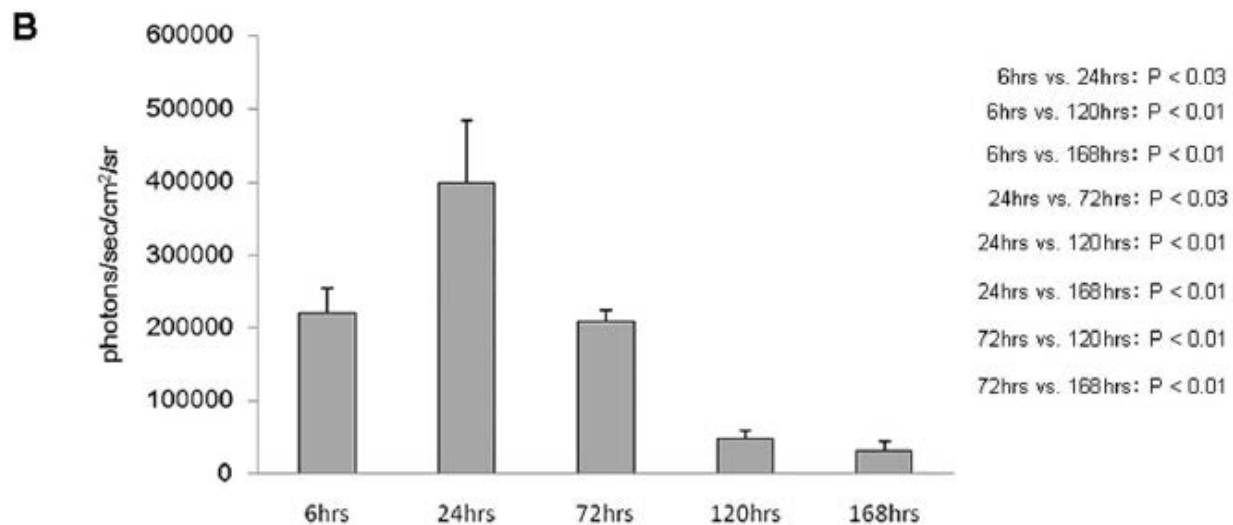
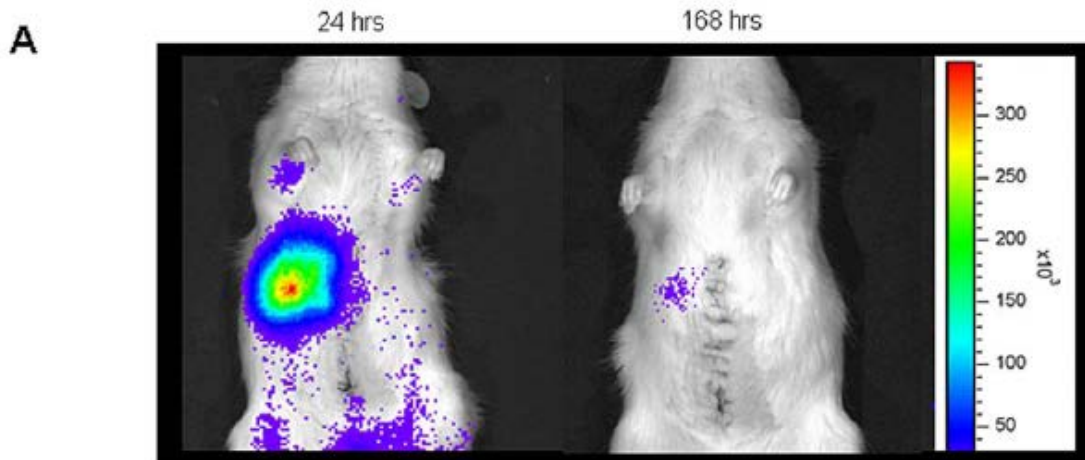


## Non-Vascularized Graft



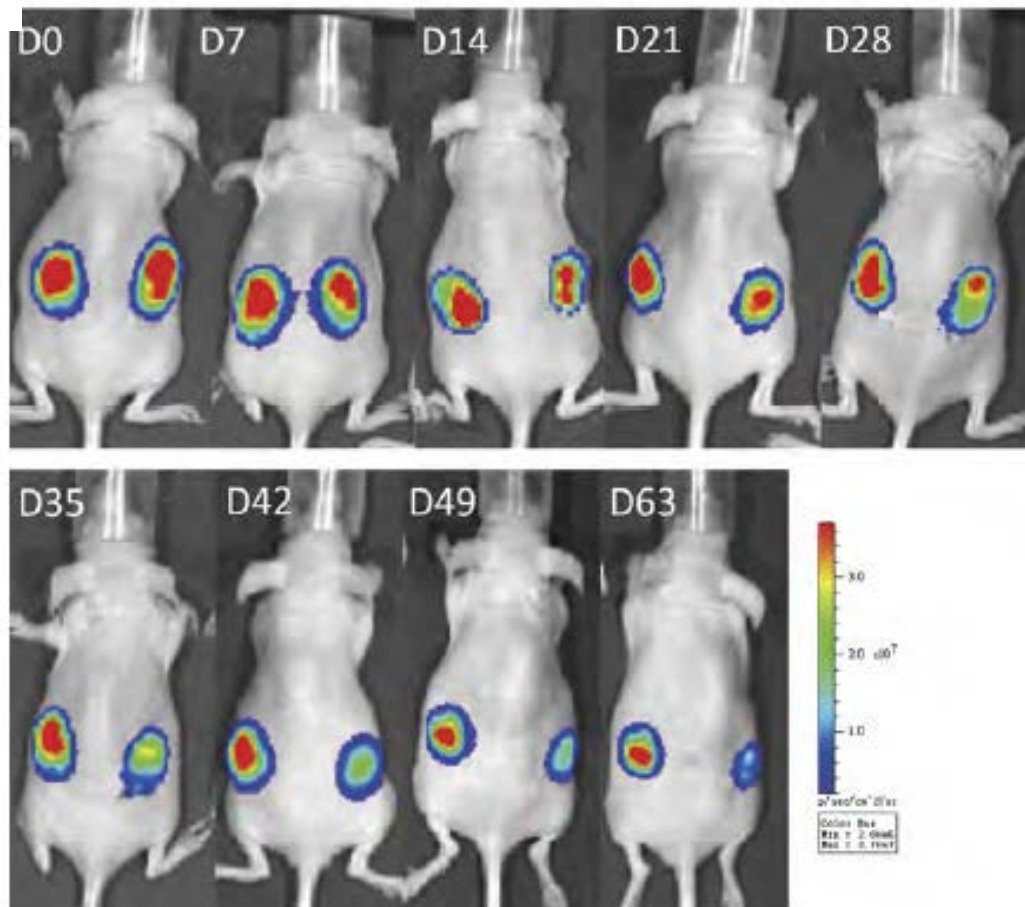
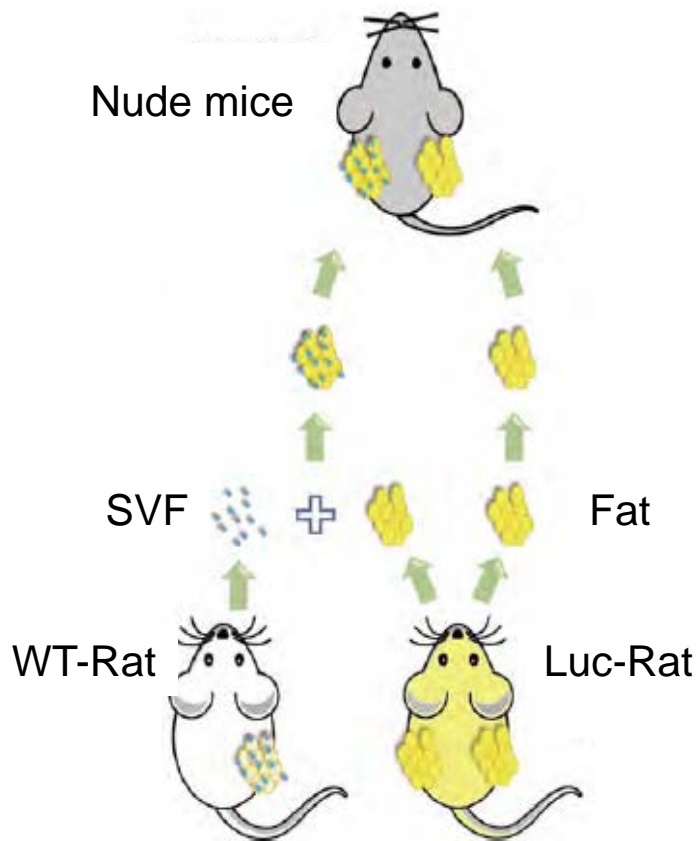
(Sunaga A, et al. *Plast Reconstr Surg Glob Open* 2013)

# Bone Marrow-Derived Mesenchymal Stem Cells Ameliorate Hepatic Ischemia Reperfusion Injury in a Rat Model



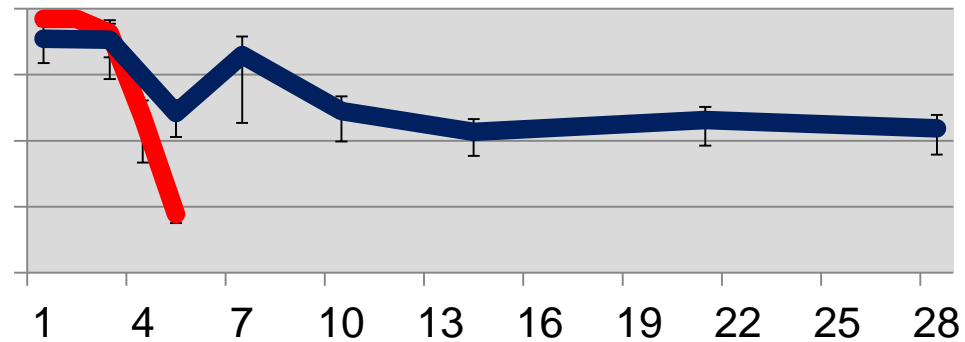
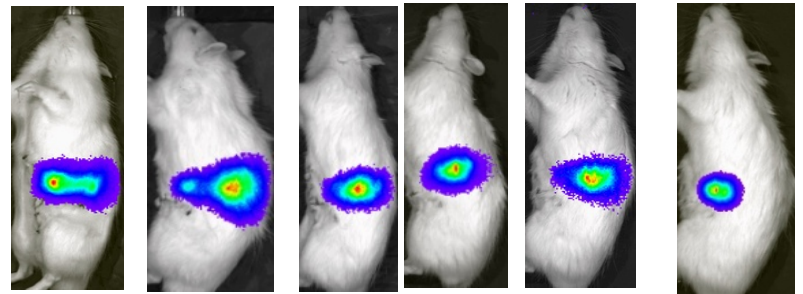
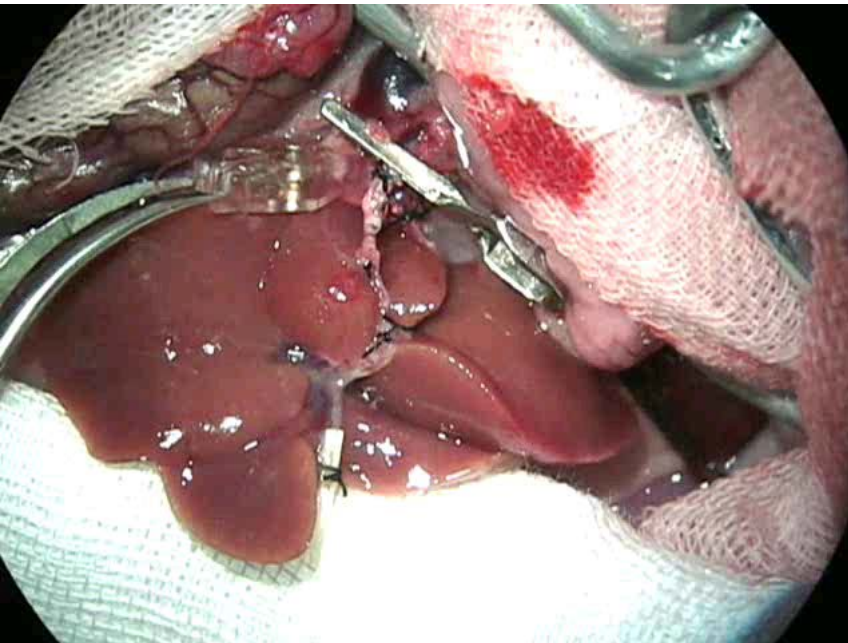
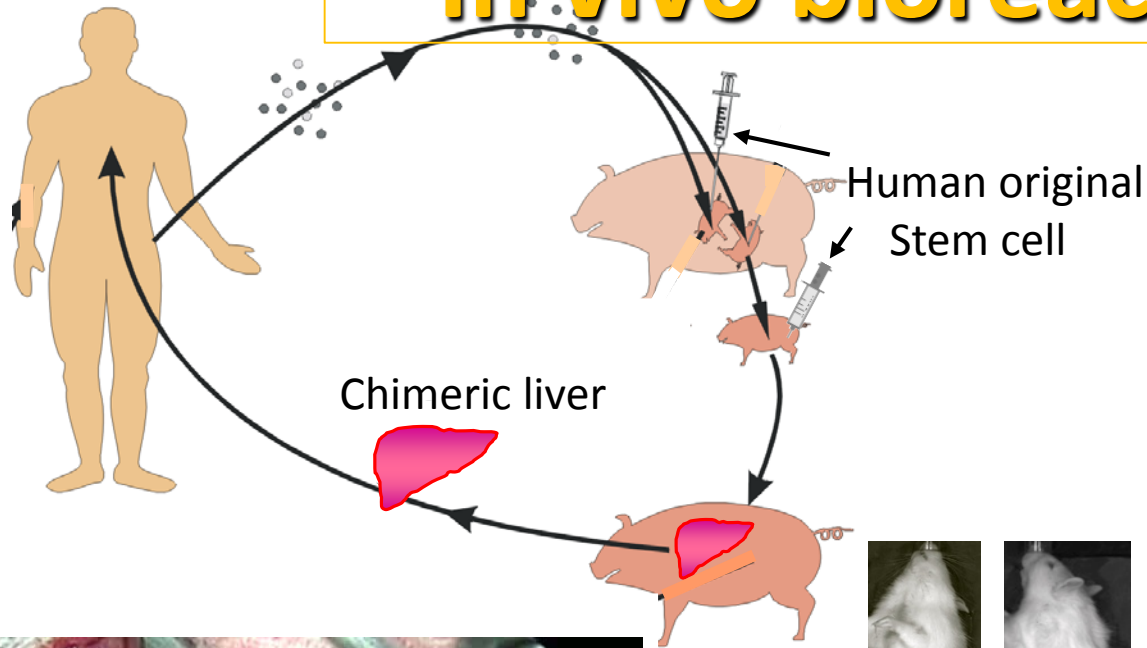
(H Kanazawa et al. PLoS One 6(4):e19195 2011)

# In Vivo Bioimaging Analysis of Stromal Vascular Fraction-Assisted Fat Grafting: The Interaction and Mutualism and Cells and Grafted Fat



(Zhou SB, et al. Transplantation 2014)

# In vivo bioreactor



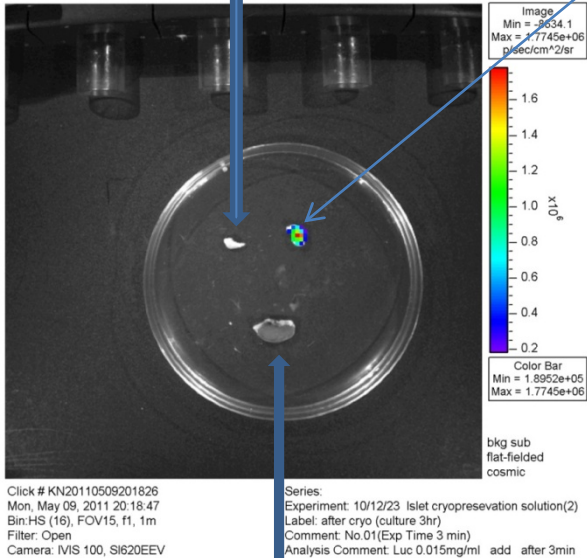
(Hata T, et al. Ann Surg 2012)



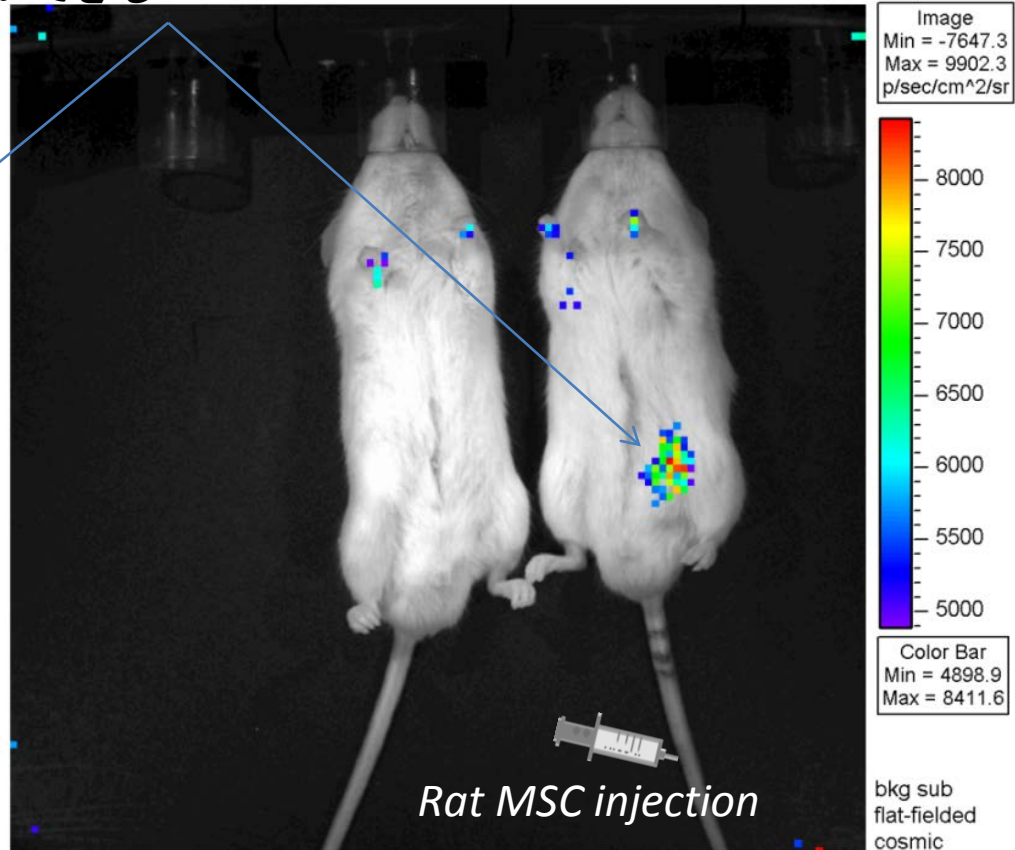
# Organ bud transplantation

繰り返しMSCを移入することで「自己化率」  
を高めることができる

Mouse  
Metanephros



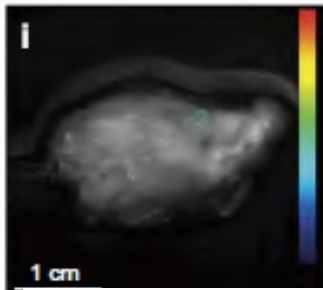
マウス腎臓



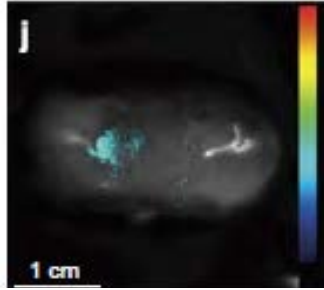
(Matsumoto K, et al. Stem cell. 2012)

# *In vitro* fabrication

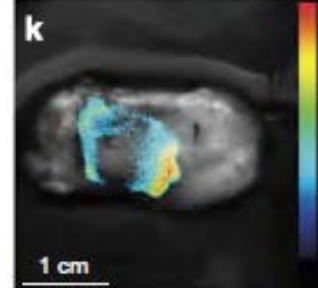
EC (-) FGF(-)



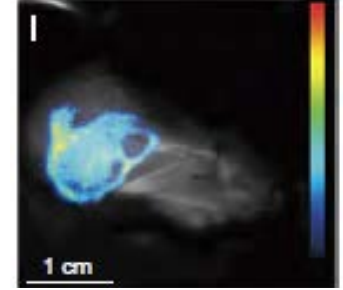
EC (-) FGF(+)



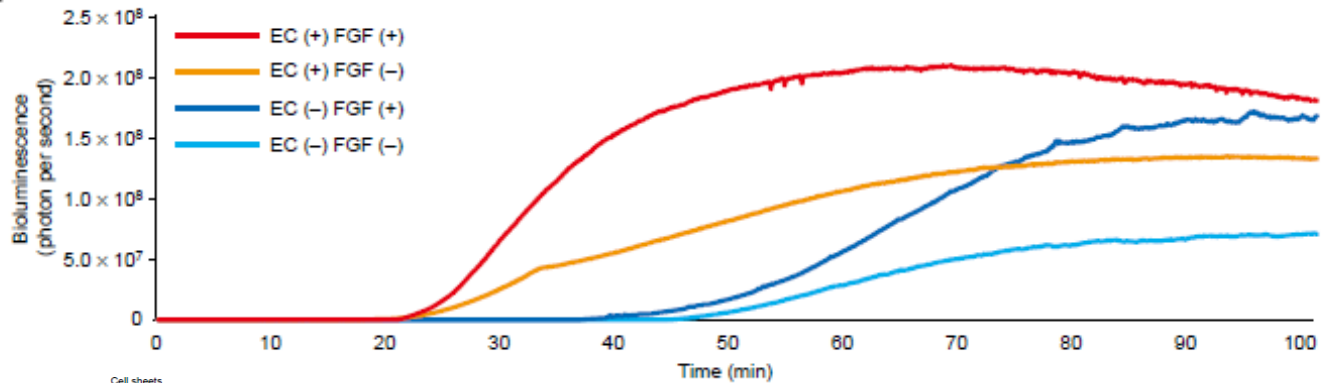
EC (+) FGF(-)



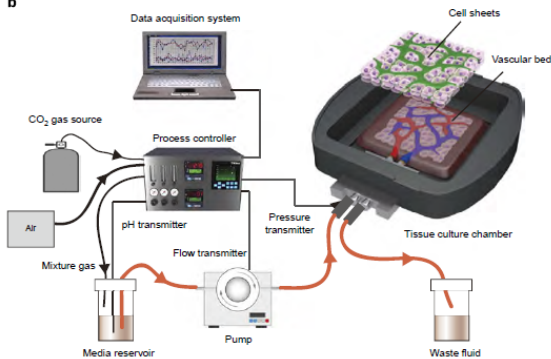
EC (+) FGF(+)



m



b

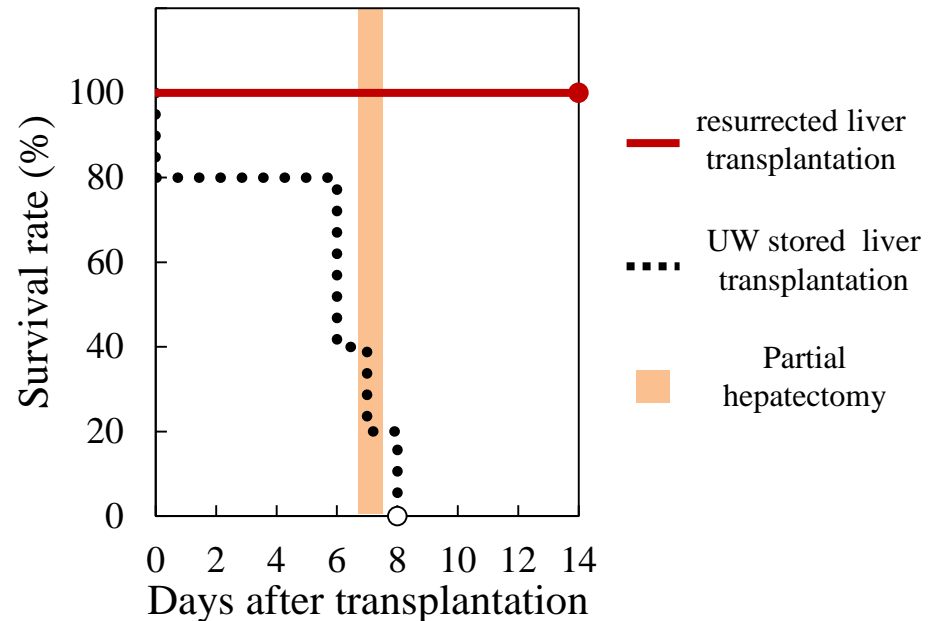
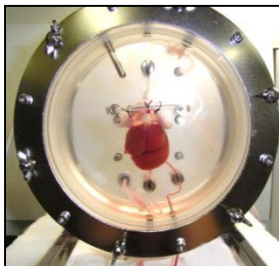
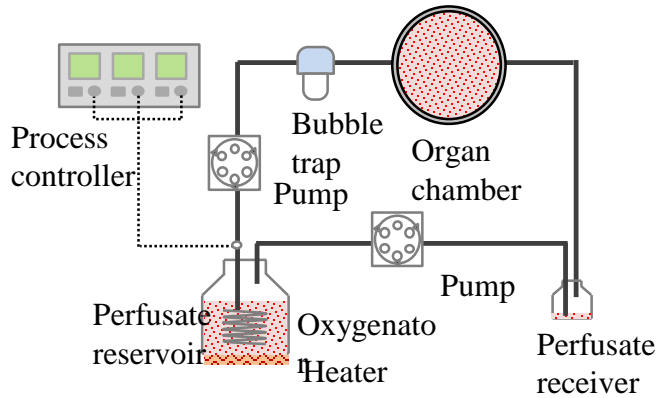
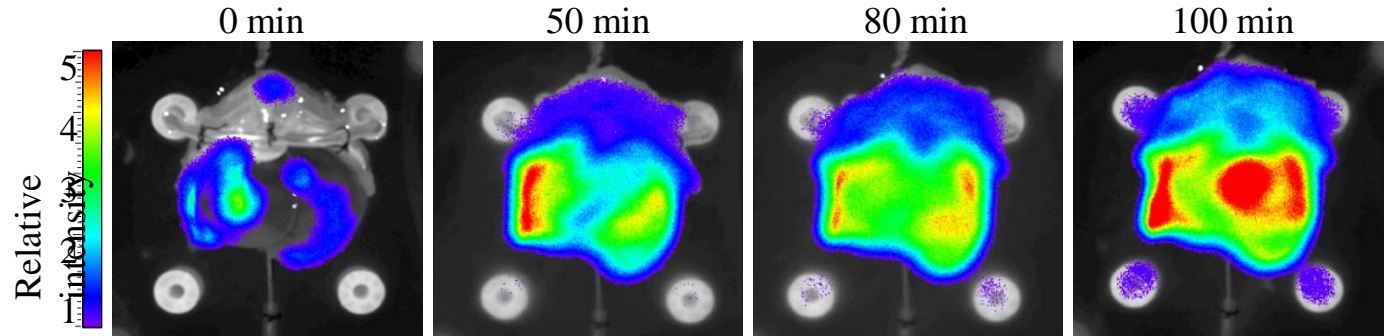


*(Sekine H, et al. Nature Communi 2013)*



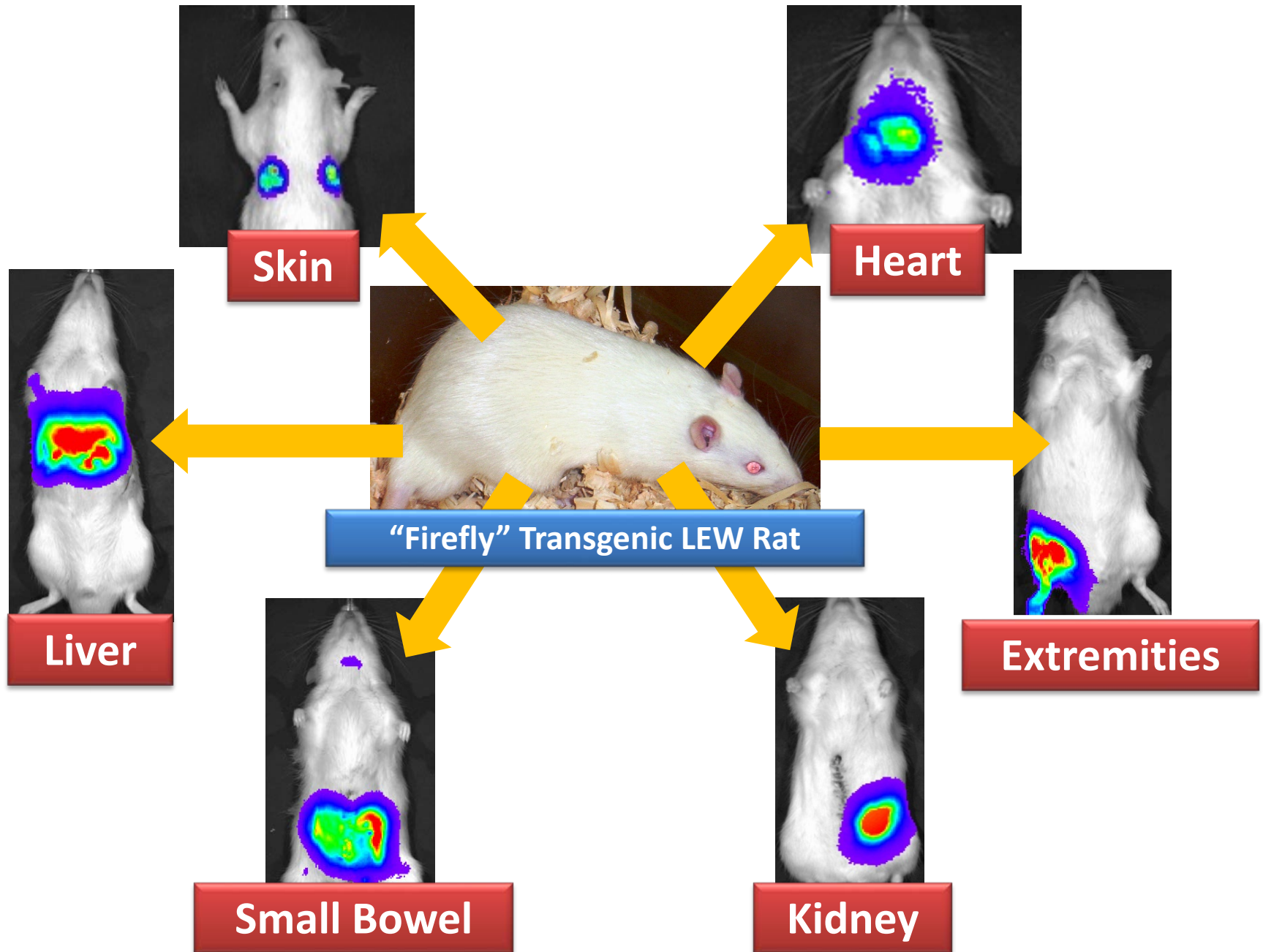
# Organ Culture

## Hypothermic temperature effects on organ survival and restoration



(Ishikawa J, et al. Scientific Reports 2015)

# Real-Time Detection System for Perfect Microsurgery





*Shall we do our best for the suffering patients*



2011/06/10 21:53