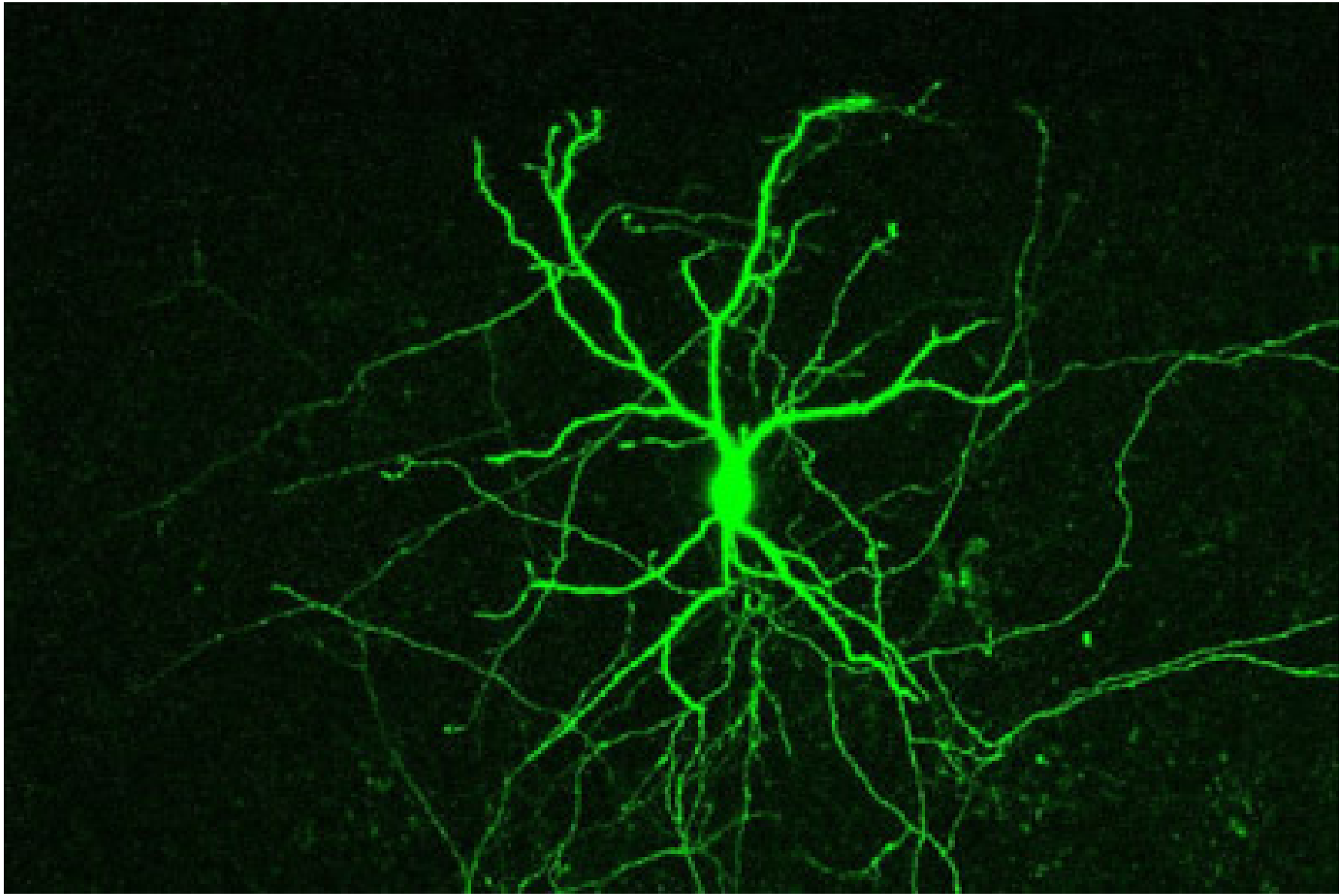
Plastics to vesicles to therapy

“I JUST WANT TO SAY ONE WORD TO YOU – JUST ONE WORD…….PLASTICS.”

Stem cells

- Types: Adult marrow
- Embryonic stem cells
- Induced Pluripotent stem cells
- Mesenchymal stem cells





MARROW CELLS TO NONMARROW TISSUE

- **Bone, cartilage, lung**
  - **Pereira et al: PNAS 92:4857,1995**
- **Skeletal muscle**
  - **Ferrari et al: Science 279:1528,1998**
- **Osteoblasts**
  - **Nilsson et al: J Exp Med 189:729,1999**
- **Neuronal**
  - **Eglitis MA, Mezey E: PNAS 94:4080,1997**

MARROW CELLS TO NONMARROW TISSUE

- **Hepatocytes**
- **Lung alveolar epithelium**

- **Theise et al: Hepatology 32:11,2000**
- **Kotton et al: Development 128: 5181,2001**

FUNCTION & QUANTITATION

- **Liver**
- **Heart**

- Lagasse et al: Nat Med 6:1229,2000
- Orlic et al: Nature 410:701,2001
- Orlic et al; PNAS 98:10344,2001

QUANTITATION & FUNCTION

- **Muscle**
  - **Gussoni et al: Nature 401:390, 1999**
- **Brain**
  - **Li Y; Neurology 56:1666,2001**

CLONAL ORIGIN OF NONHEMATOPOIETIC CELLS FROM MARROW

- **Krause DS et al: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105: 406, 2000**

CLONAL ORIGIN OF NONHEMATOPOIETIC CELLS FROM MARROW

- **Krause DS et al: Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 105: 406, 2000**

An Unnecessary and Unseemly Debate

- Components
- Far religious right
- Embryonic stem cell investigators
- Adult stem cell investigators
- Commercial interests, read Biotech
- Ego and fame –read “stockholm fever”
- Patient advocate groups

Sequence

- The religious right sees the adult stem cell results with heart and liver studies in mice and concludes that human embryonic research is unnecessary.
- Bush regulations
- Embryonic stem cell researchers driven by belief, greed, ego and $$$$$ attack the adult stem cell field.They are supported by patient advocate groups.

BioPharma and Biotech

- **No ethical problems here – these folks are after money and are comfortably amoral. That OK, they are businesses, but they try to claim otherwise.**
- **Watch out about ultimate goals in the cloning area.**

Ignoratio Elenchi

- Red Herrings or irrelevant conclusions
- The phenomena must be demonstrated on a clonal basis.
- It must be “ROBUST”
- It must not represent fusion
- Function must be shown on an organ level

Grant Reviewer

Editor





Wise Researcher



Confused Academic





Transdifferentiation

Clonal

Robust

Fusion

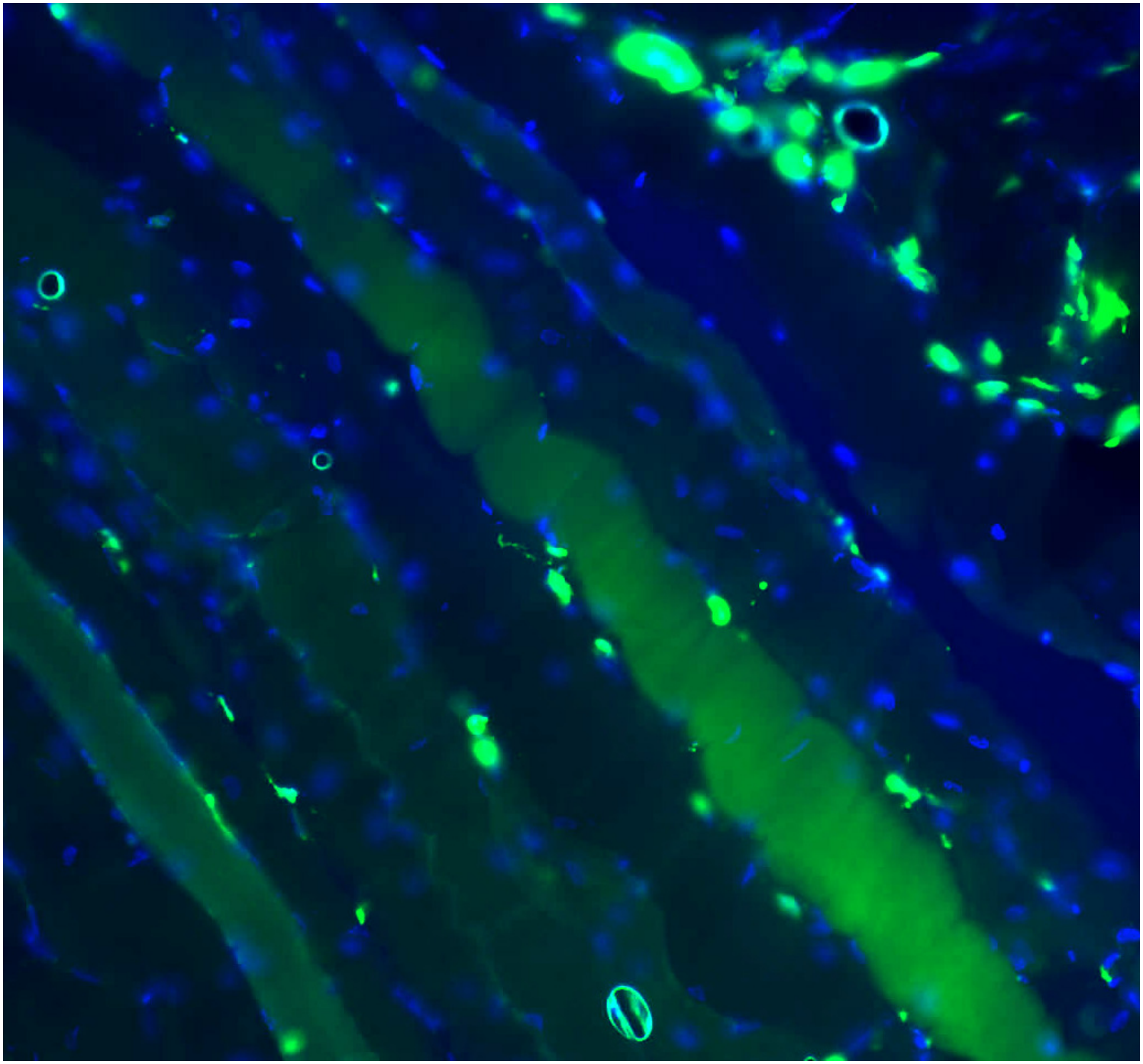


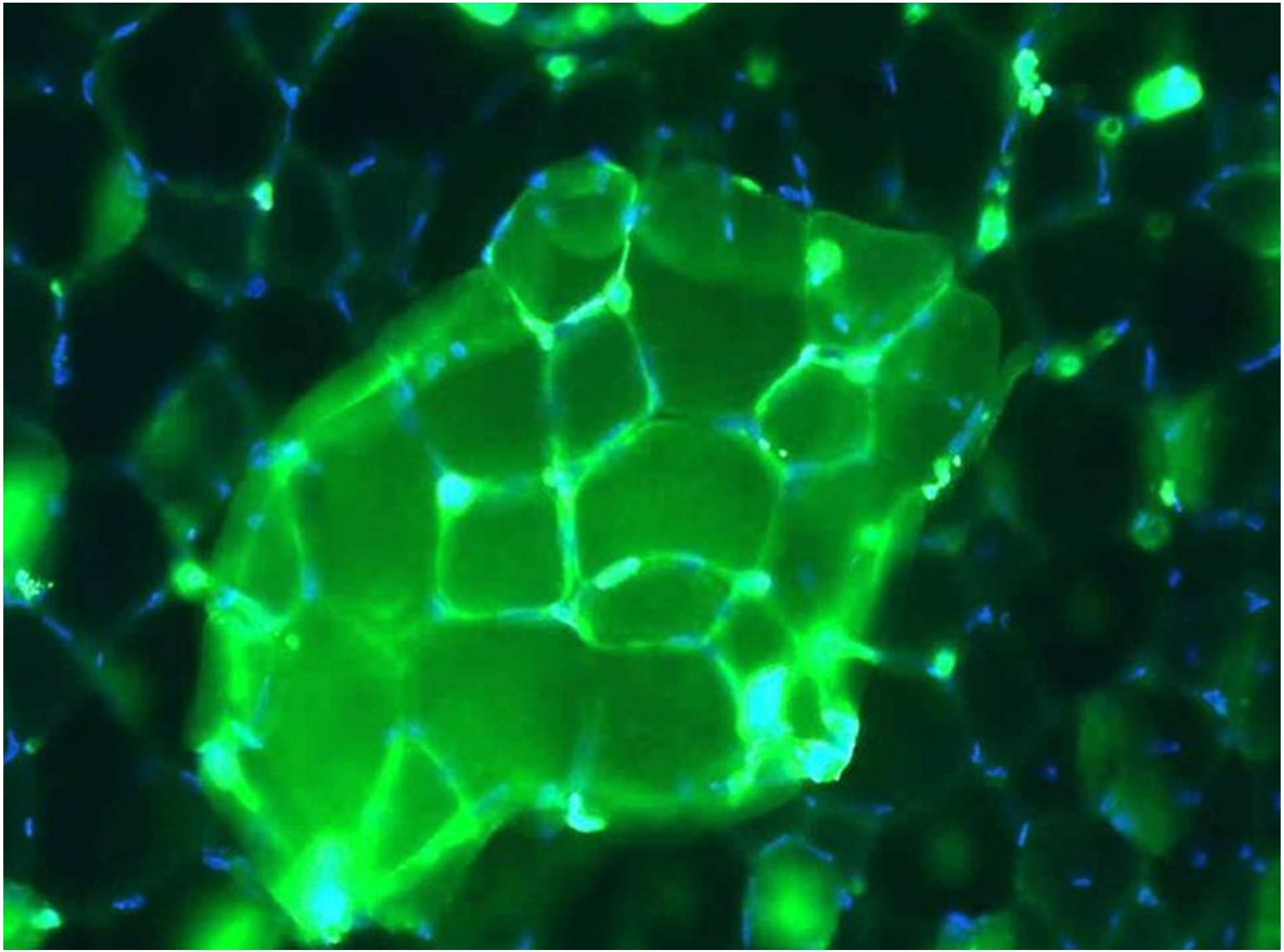
n cell tissue restoration

Results of this Divisive debate

- Difficulty with publishing
- Difficulty with grant funding
- Young investigators leaving the field

Skeletal muscle production from adult marrow cells





Cardiotoxin Injury

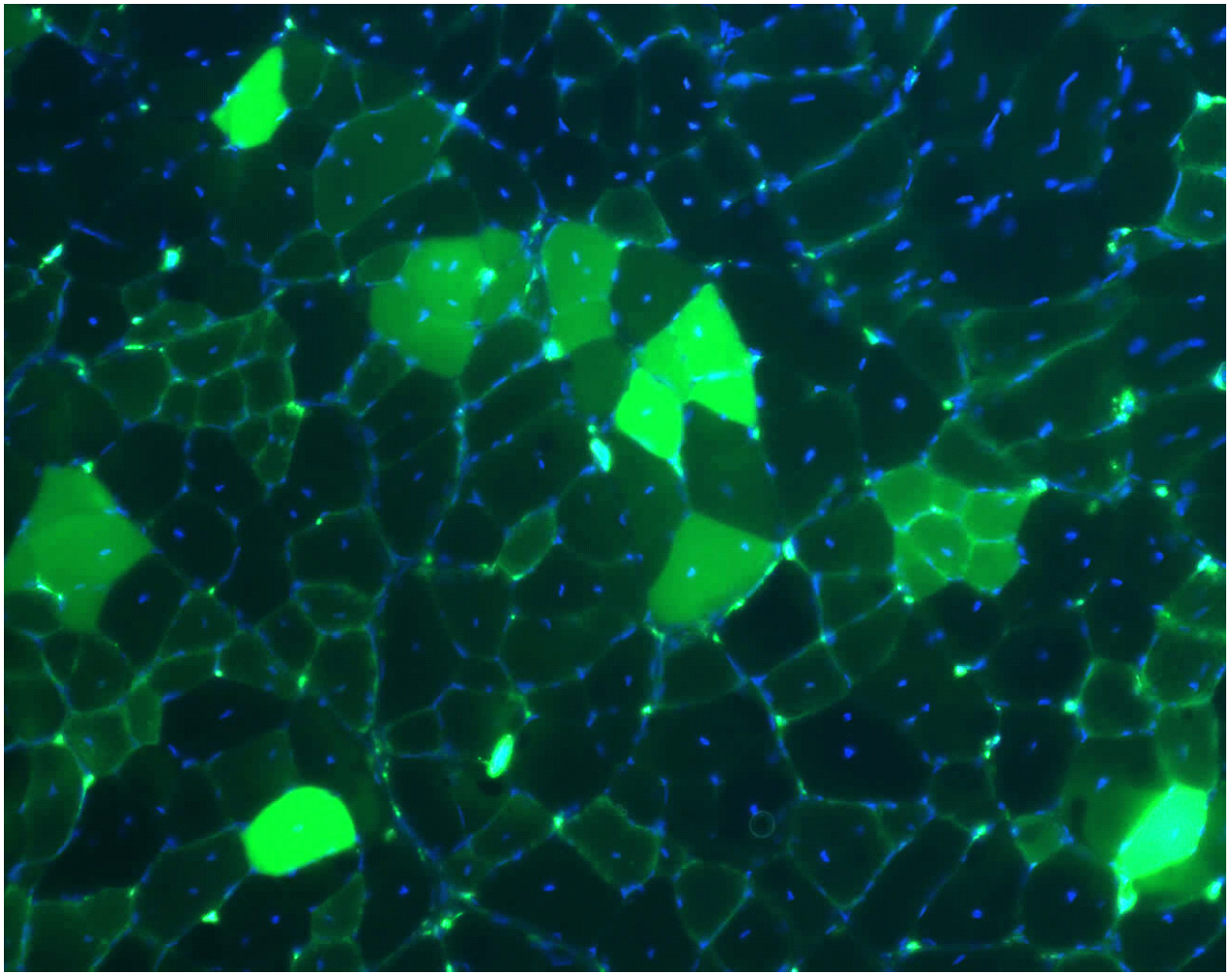
Radiation

Cell dose

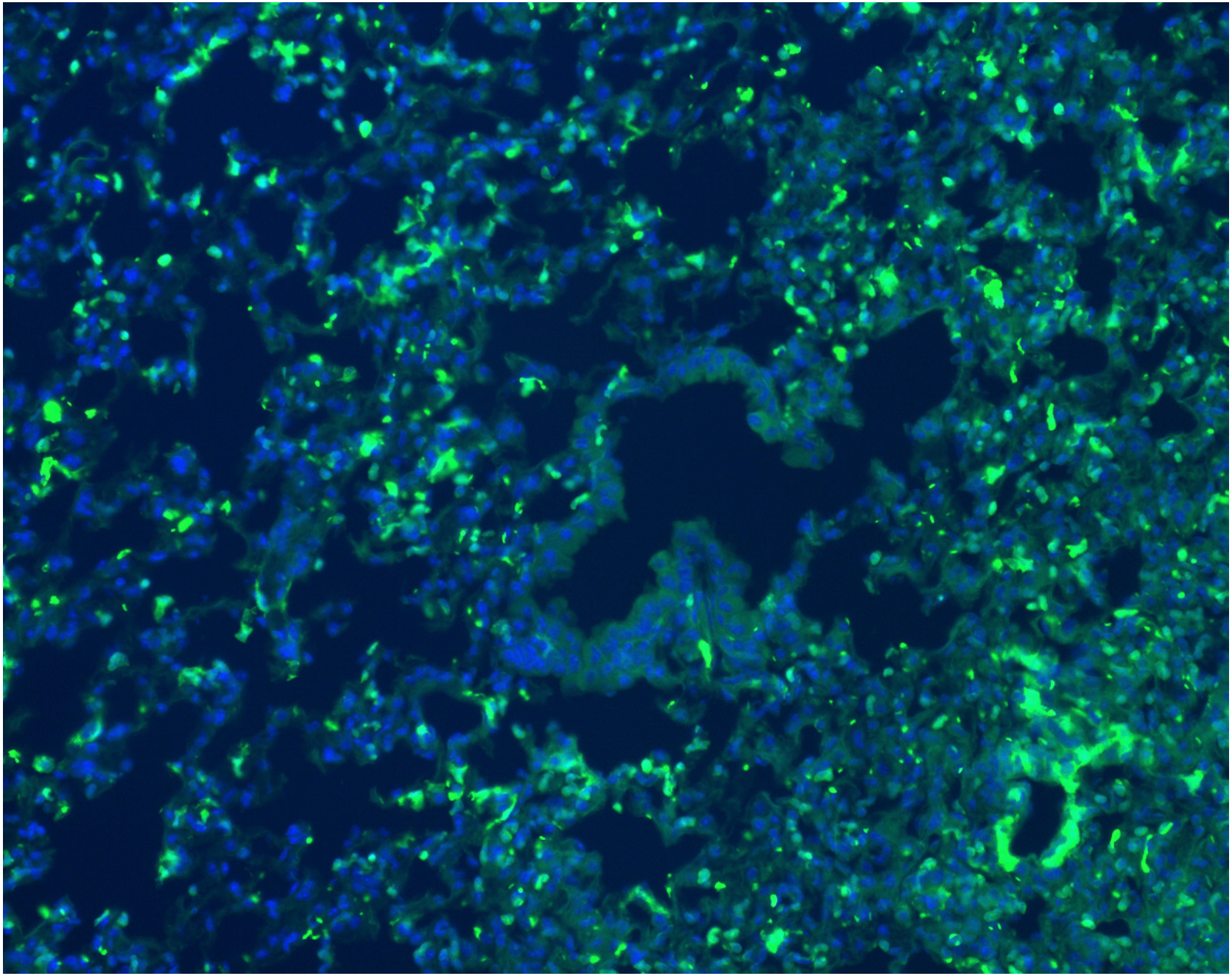
Cell Type

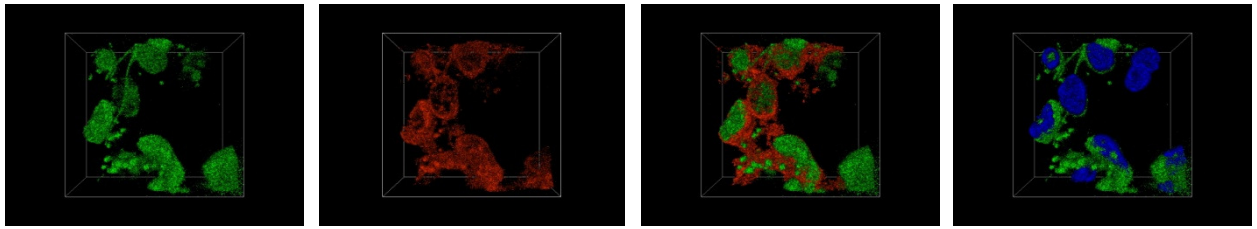
Mobilization

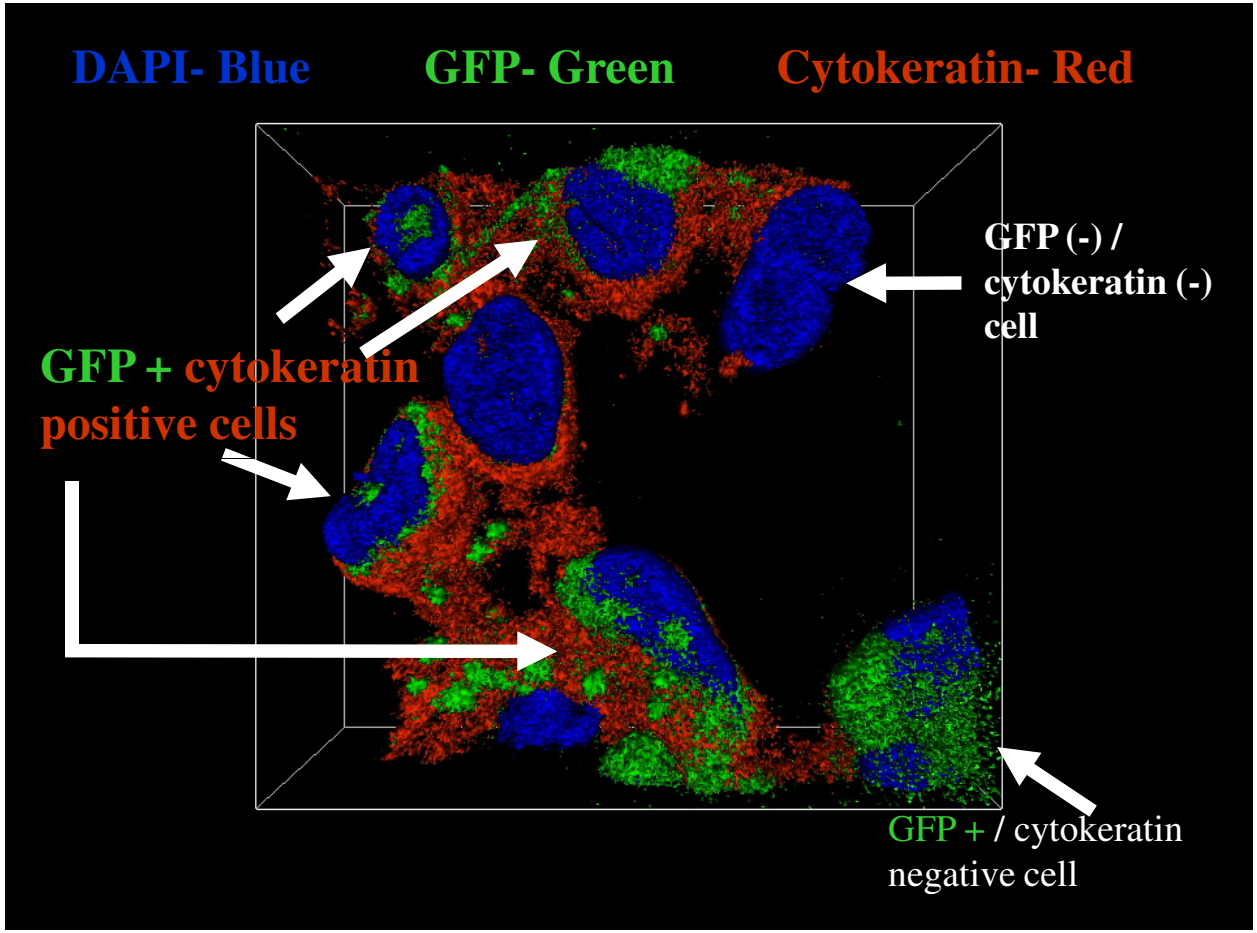
Time of injury

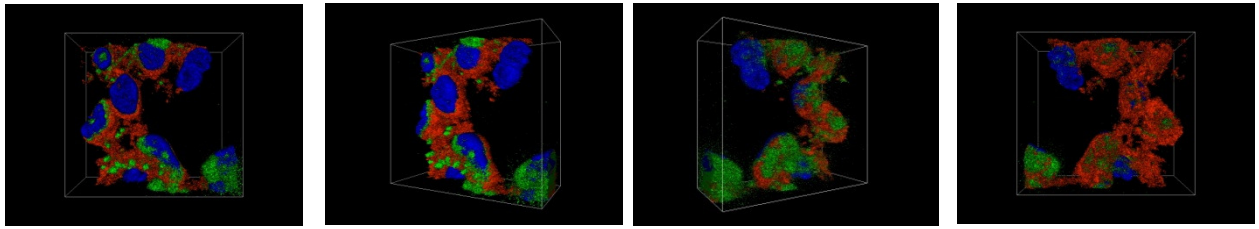


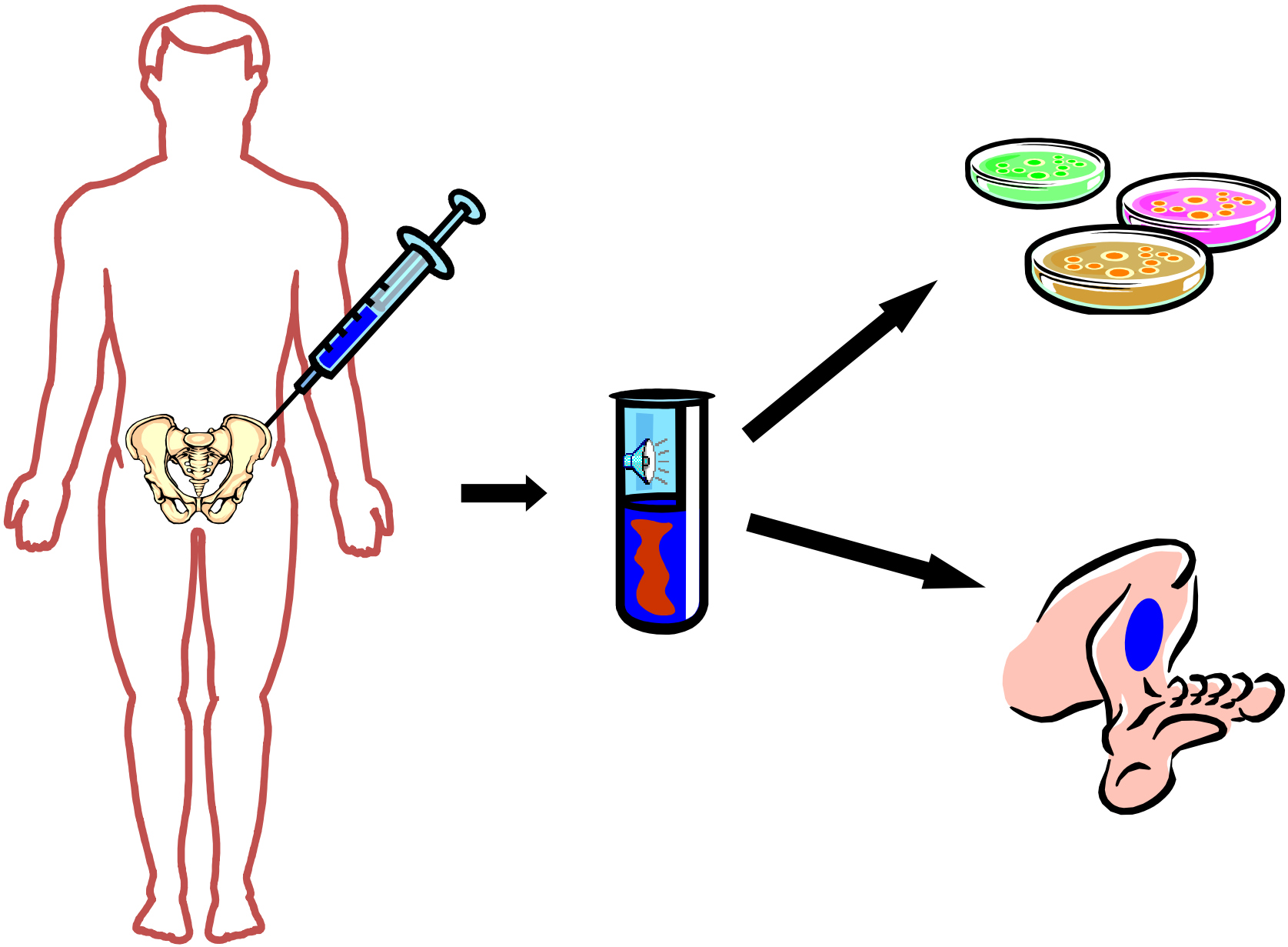
Marrow Conversions to Lung

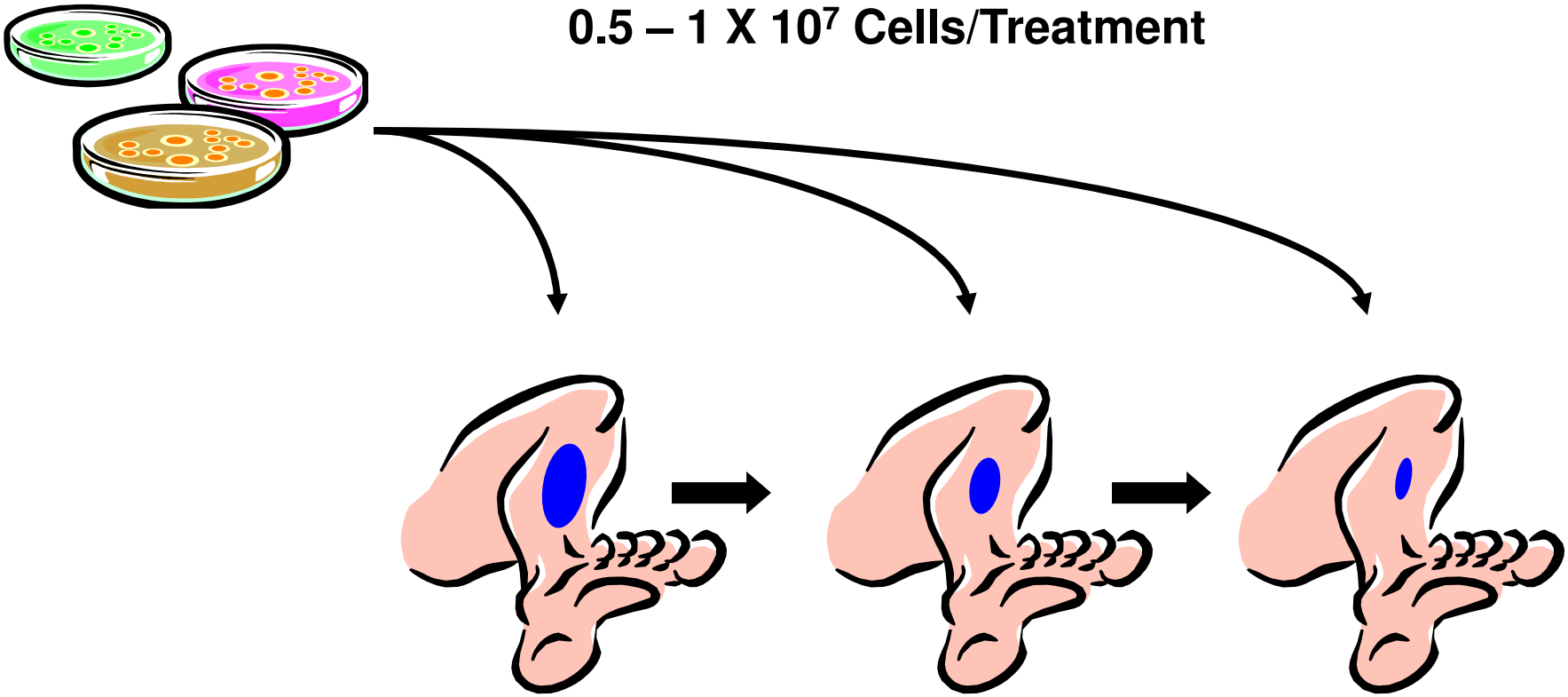





DAPI- Blue
GFP- Green
Cytokeratin- Red
GFP (-) / cytokeratin (-) cell
GFP + cytokeratin positive cells
GFP + / cytokeratin negative cell






0.5 – 1 X 10^7 Cells/Treatment







Treatment with Marrow Cells

- **An 83 year old lady with inoperable arterial insufficiency and a necrotic wound with exposed Achilles tendon.**
- **Chronic ulceration over one year in duration**
- **Loss of tendon was considered certain and amputation was being considered.**
- **Wound size 11X4cm**

Treatment with Bone Marrow

- **Fresh marrow initially to wound and wound edges.**
- **Cultured marrow given three times; 11, 6 and 10 million cells into the wound**
- **Each application of cultured marrow led to a burst of granulation tissue and epithelialization and 4 months after the last marrow application the wound completely healed**



Treatment with Bone Marrow

- **81year old lady with a nonhealing venous ulcer complicated by arterial insufficency of over one year's duration.**
- **One application of fresh marrow and one of culture marrow (5.2 million cells)**
- **Within 2 weeks after first administrationof cultured marrow the wound was completely healed and has remained so for over one year**



How do marrow cells make non marrow cells?

Microvesicles

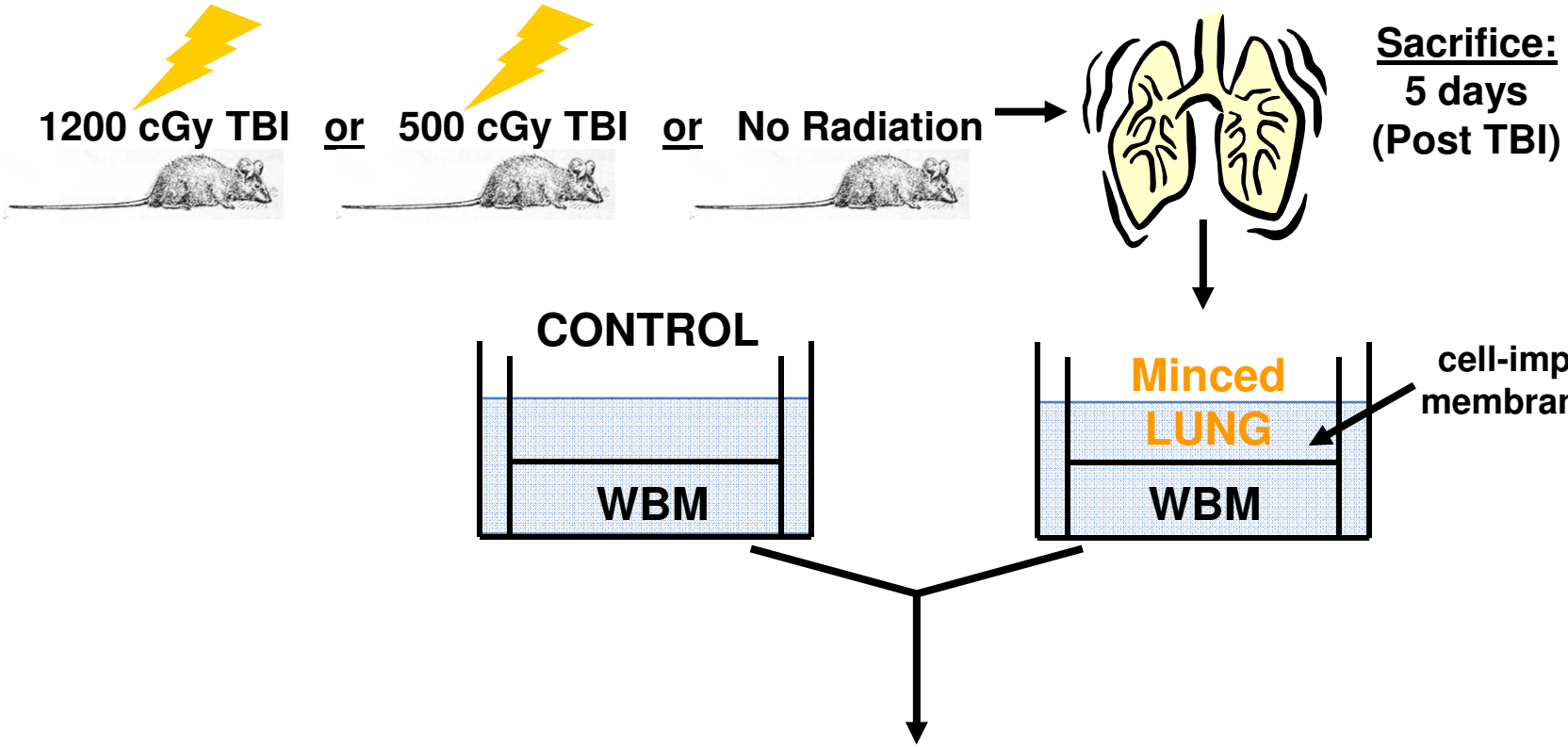
- **A mechanism for cell fate change**

History

- **Cell junk – platelets and erythrocytes**
- **Vaccines**
- **Modulate cell phenotype**

Experimental Design

Lung / Bone Marrow Cell Co-Cultures



Real Time RT-PCR on co-cultured WBM

Pulmonary epithelial cell genes

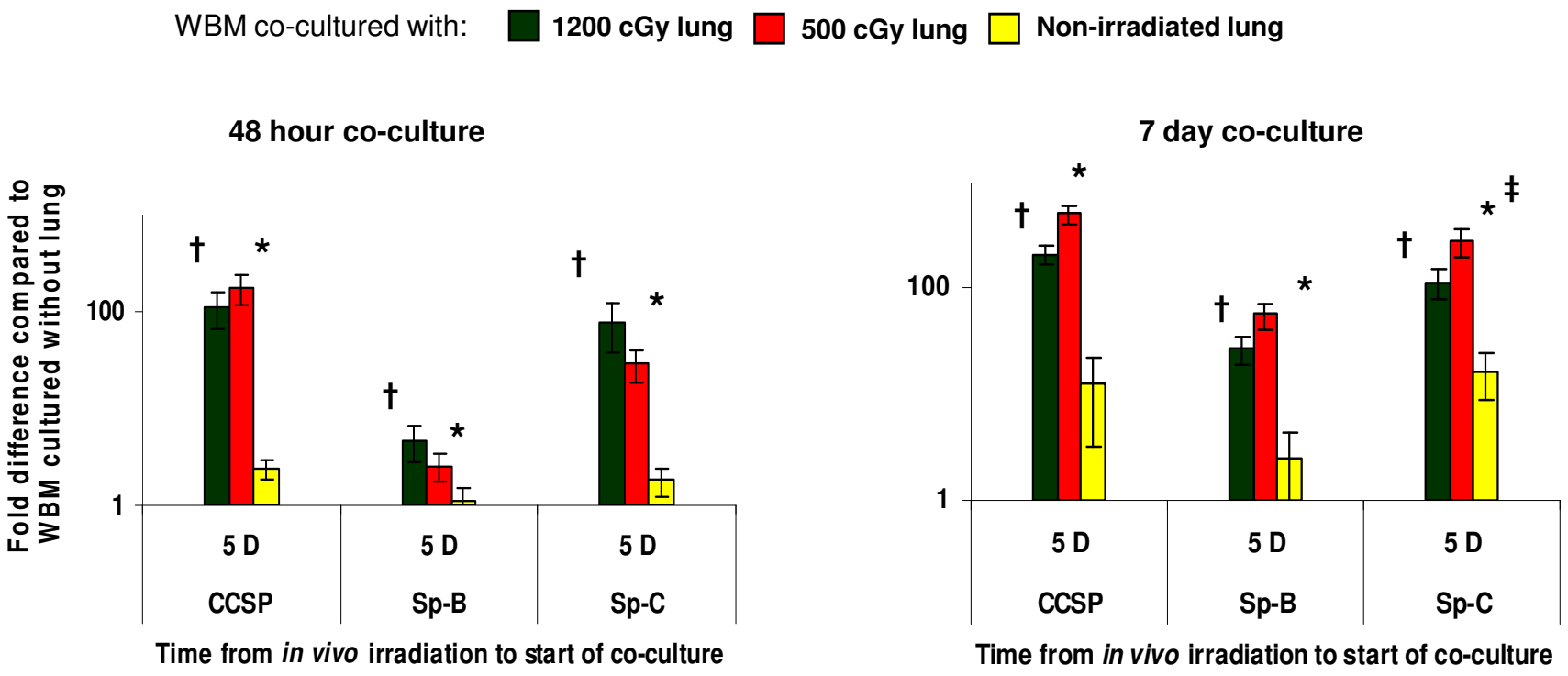
Type I pneumocyte: Aquaporin-5

Type II pneumocyte: Sp-A, B, C, D

Clara cell: CCSP

WBM cultured *without* lung (control)

Marrow cells co-cultured with lung have increased pulmonary epithelial cell mRNA expression



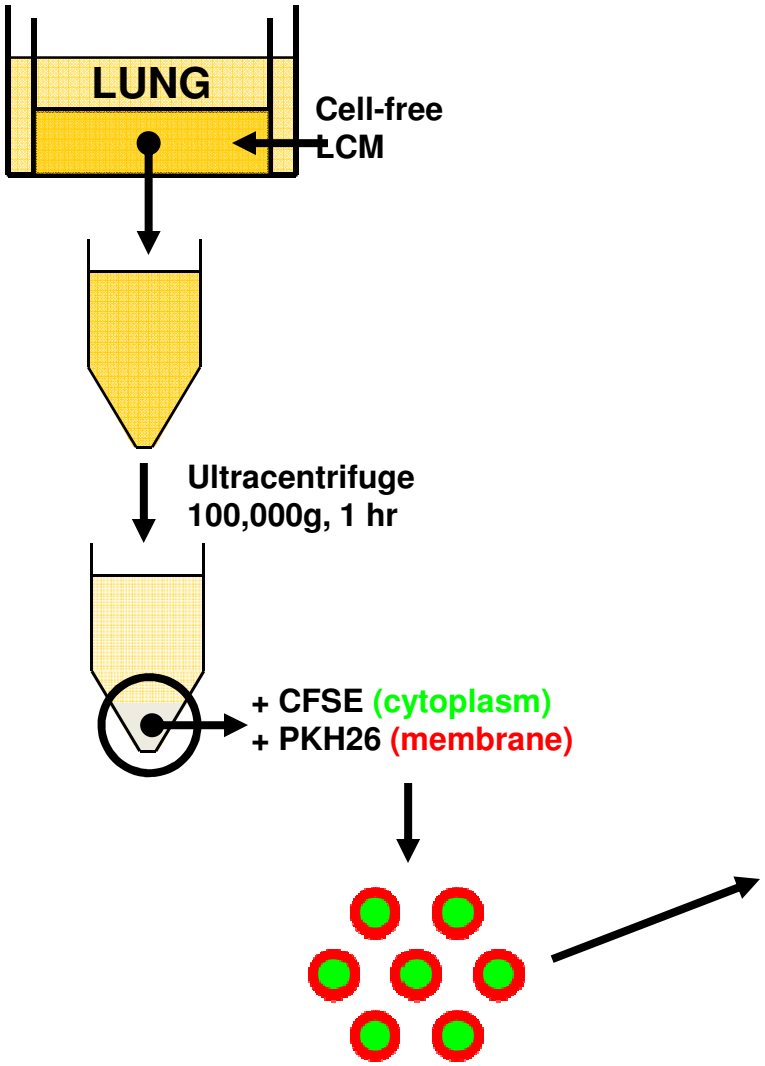
* 500 cGy lung/WBM vs. non-irradiated lung/WBM co-culture groups: Wilcoxon p=0.01, n=9.

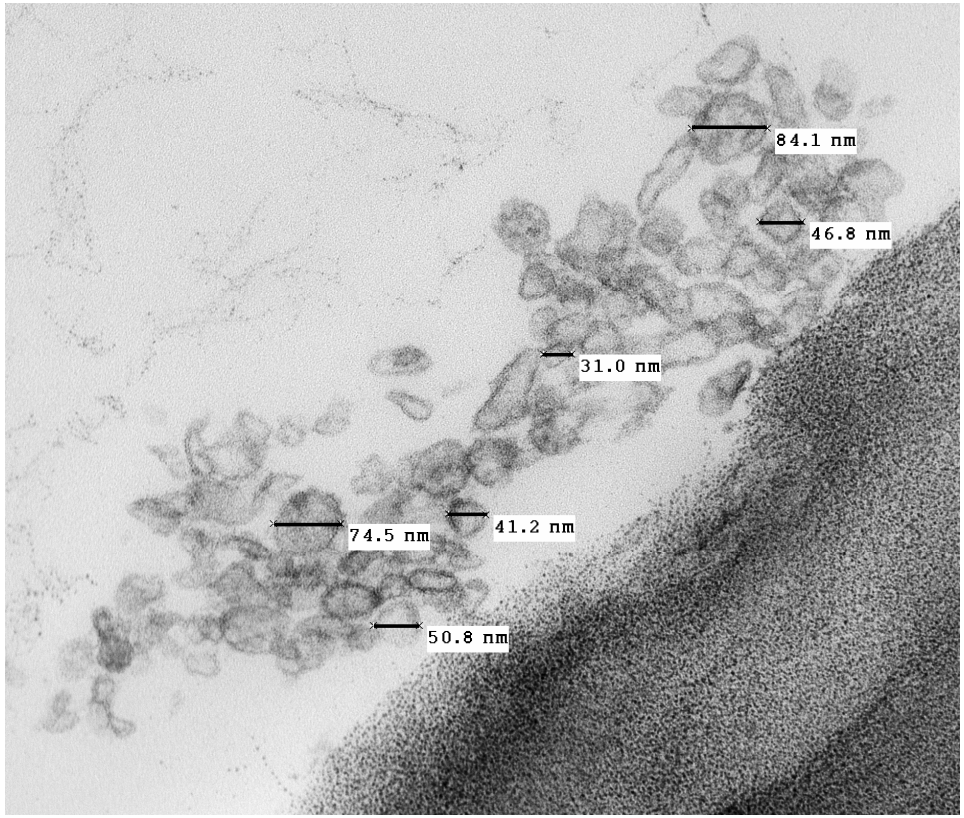
†1200 cGy lung/WBM vs. non-irradiated lung/WBM co-culture groups: Wilcoxon p=0.01, n=9.

‡ 500 cGy lung/WBM vs. 1200 cGy/WBM co-culture groups: Wilcoxon p=0.01, n=9

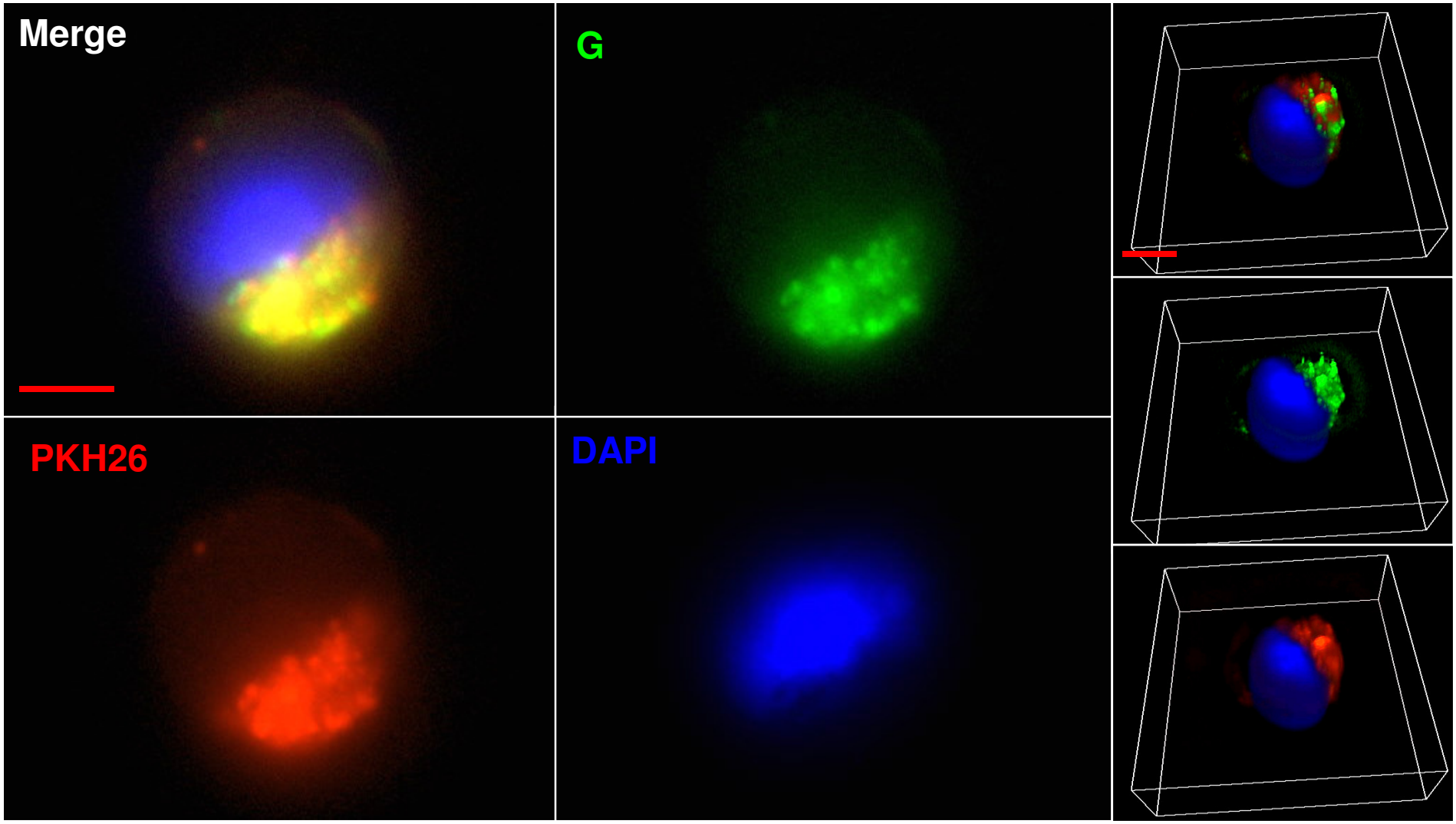
From Aliotta JM et al., Stem Cells 2007

Microvesicles can be isolated from lung conditioned media



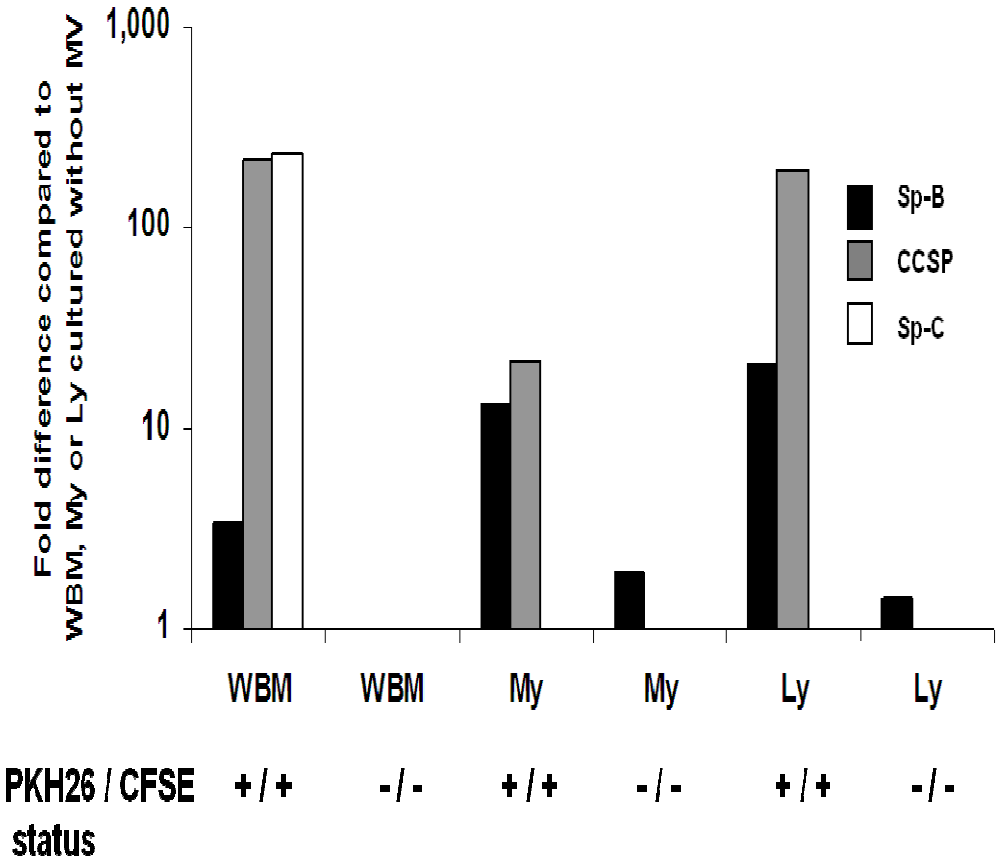


From Aliotta et al. Exp Hematol. 2010;38(3):233-245

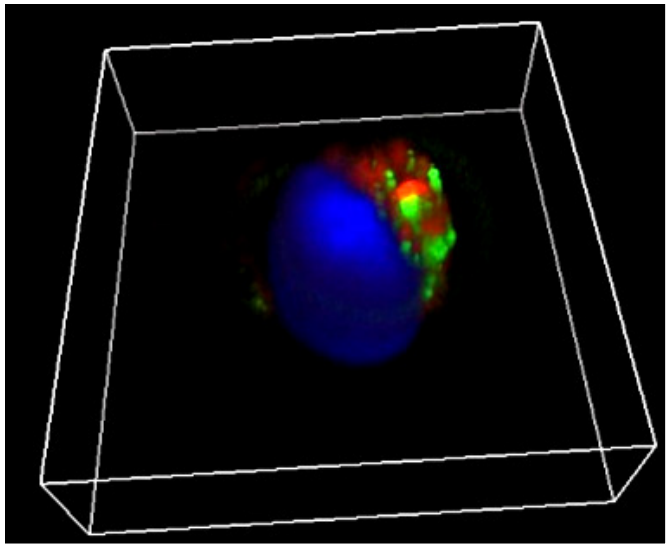


Red Bar = 10μm

Effect of internalized microvesicles



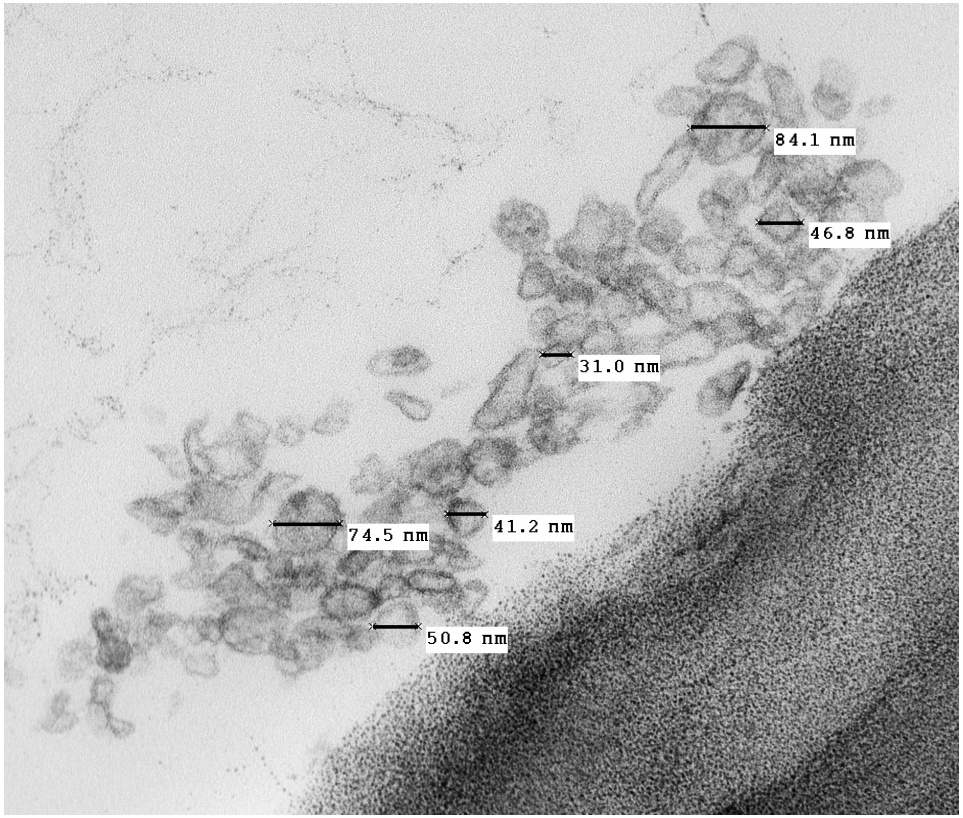
Pulmonary epithelial cell mRNA expression



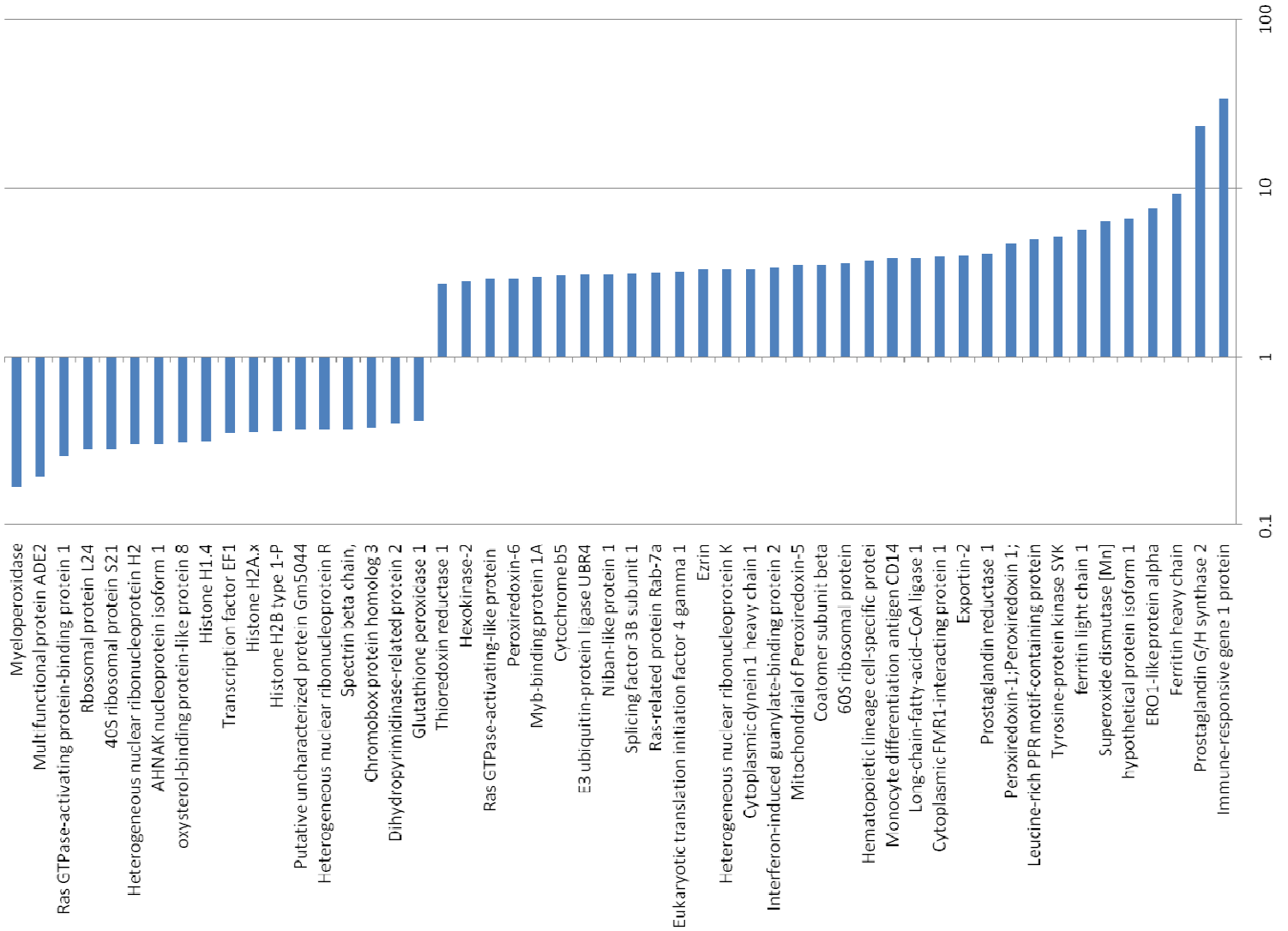
From Aliotta et al. Exp Hematol. 2010;38(3):233-245

Contents of lung-derived microvesicles

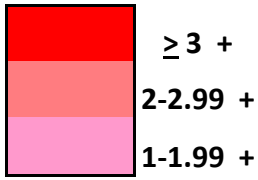
- mRNA
  - Derived from pulmonary epithelium
- Non-coding RNA
  - >200 different species miRNA
  - Long non coding RNA
- Protein
  - Adhesion, transcription
  - Quantitative / qualitative differences (radiation-injured vs. normal)
- DNA
  - Genomic, mitochondrial



Altered proteome of microvesicle-modified marrow cells



Altered miRNA profile of microvesicle-modified marrow cells



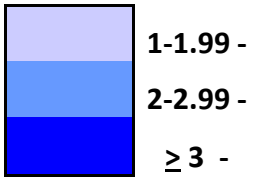
Unregulated miRNA species (101)

mmu-miR-720
mmu-miR-125b-5p
mmu-miR-199a-3p
mmu-miR-199b
mmu-miR-143
mmu-miR-142-5p
mmu-miR-770-5p
mmu-miR-16
mmu-miR-223
mmu-miR-15b
mmu-miR-142-3p
mmu-miR-27a
mmu-miR-27b
mmu-let-7a
mmu-let-7f
mmu-miR-19b
mmu-miR-487b
mmu-miR-140*
mmu-miR-30b
mmu-miR-29b
mmu-miR-29a
mmu-miR-195
mmu-let-7b
mmu-miR-26a
mmu-miR-30c
mmu-miR-24
mmu-miR-106b
mmu-let-7g
mmu-miR-690
mmu-miR-30d
mmu-miR-30a
mmu-miR-29c
mmu-miR-20a

mmu-miR-17
mmu-miR-15a
mmu-miR-451
mmu-miR-106a
mmu-miR-494
mmu-let-7i
mmu-let-7d
mmu-miR-23a
mmu-miR-23b
mmu-miR-30e
mmu-miR-93
mmu-miR-22
mmu-miR-103
mmu-miR-107
mmu-miR-21
mmu-miR-181a
mmu-miR-25
mmu-miR-26b
mmu-miR-574-5p
mmu-miR-466f-3p
mmu-miR-19a
mmu-miR-342-3p
mmu-miR-191
mmu-miR-34a
mmu-miR-425
mmu-miR-365
mmu-miR-140
mmu-let-7e
mmu-miR-92a
mmu-miR-130a
mmu-miR-489
mmu-miR-340-5p
mmu-miR-221

mmu-miR-301a
mmu-miR-148a
mmu-miR-212
mmu-miR-374
mmu-miR-152
mmu-miR-220
mmu-miR-208b
mmu-miR-27b*
mmu-miR-361
mmu-miR-652
mmu-miR-322
mmu-miR-328
mmu-miR-130b
mmu-miR-295*
mmu-miR-484
mmu-miR-10a
mmu-miR-125a-5p
mmu-miR-574-3p
mmu-miR-210
mmu-miR-151-5p
mmu-miR-18a
mmu-miR-378
mmu-miR-339-5p
mmu-miR-145
mmu-miR-181b
mmu-miR-193
mmu-miR-350
mmu-miR-301b
mmu-miR-21*
mmu-miR-17*
mmu-miR-338-5p
mmu-miR-500
mmu-miR-362-3p

Downregulated miRNA species (159)



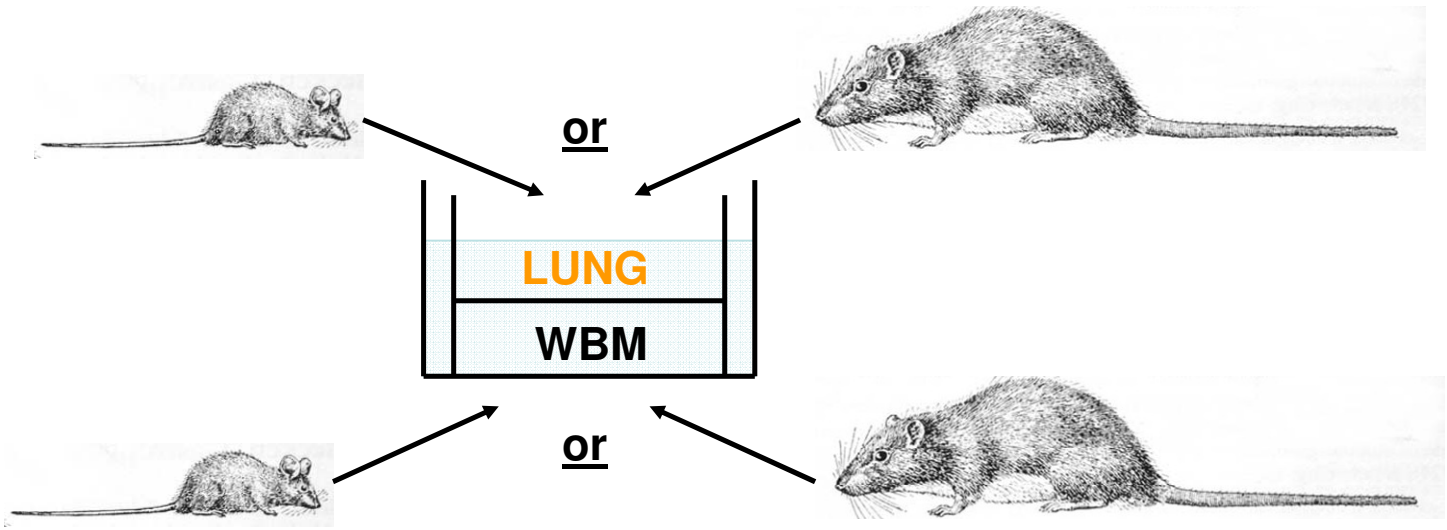
mmu-miR-376b
mmu-miR-204
mmu-miR-211
mmu-miR-710
mmu-miR-615-3p
mmu-miR-30c-1*
mmu-miR-875-3p
mmu-miR-486
mmu-miR-186*
mmu-miR-705
mmu-miR-764-5p
mmu-miR-1224
mmu-miR-762
mmu-miR-431*
mmu-miR-345-3p
mmu-miR-615-5p
mmu-miR-296-5p
mmu-miR-146b*
mmu-miR-331-3p
mmu-miR-712*
mmu-miR-701
mmu-miR-683
mmu-miR-695
mmu-miR-488*
mmu-miR-671-5p
mmu-miR-155
mmu-miR-10b
mmu-miR-433*
mmu-miR-551b
mmu-miR-665
mmu-miR-582-3p
mmu-miR-711
mmu-miR-129-3p

mmu-miR-15b*
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mmu-miR-590-5p
mmu-miR-146a
mmu-miR-105
mmu-miR-676
mmu-miR-341
mmu-miR-678
mmu-miR-345-5p
mmu-miR-412
mmu-miR-325*
mmu-miR-677
mmu-miR-714
mmu-miR-135a*
mmu-miR-483
mmu-miR-470*
mmu-miR-374*
mmu-miR-541
mmu-miR-323-5p
mmu-miR-691
mmu-miR-294*
mmu-miR-124
mmu-miR-497
mmu-miR-680
mmu-miR-139-3p

mmu-miR-127*
mmu-miR-708
mmu-miR-760
mmu-miR-485
mmu-miR-670
mmu-miR-337-3p
mmu-miR-30c-2*
mmu-miR-675-5p
mmu-miR-493
mmu-miR-199b*
mmu-miR-434-5p
mmu-miR-423-3p
mmu-miR-542-5p
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mmu-miR-146b
mmu-miR-337-5p
mmu-miR-125b-3p
mmu-miR-877
mmu-miR-290-5p
mmu-miR-491
mmu-miR-290-3p
mmu-miR-106b*
mmu-miR-539
mmu-miR-450a-3p
mmu-miR-470
mmu-miR-744
mmu-miR-200c*
mmu-miR-15a*
mmu-miR-191*
mmu-miR-453
mmu-miR-150*
mmu-miR-370
mmu-miR-188-5p
mmu-miR-763

mmu-miR-485*
mmu-miR-292-3p
mmu-miR-181a-2*
mmu-miR-873
mmu-miR-188-3p
mmu-miR-183*
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mmu-miR-743b-3p
mmu-miR-138*
mmu-miR-465a-3p
mmu-miR-465b-3p
mmu-miR-465c-3p
mmu-miR-423-5p
mmu-miR-879*
mmu-miR-742
mmu-miR-801
mmu-miR-133a*
mmu-miR-382
mmu-miR-715
mmu-miR-138
mmu-miR-876-3p
mmu-miR-302c*
mmu-miR-199a-5p
mmu-miR-674
mmu-miR-330*
mmu-miR-666-5p
mmu-miR-874
mmu-miR-196a*
mmu-miR-292-5p
mmu-miR-331-5p
mmu-miR-30b*
mmu-miR-218-2*
mmu-miR-28

Experimental Design
Rat / Mouse Co-Cultures



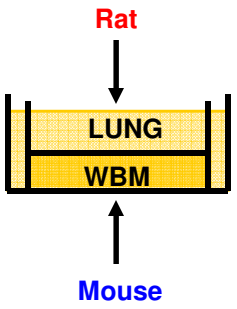
RT-PCR on co-cultured WBM cells (7 days)
using **species-specific** Sp-B primers

Example: Murine WBM co-cultured with rat lung

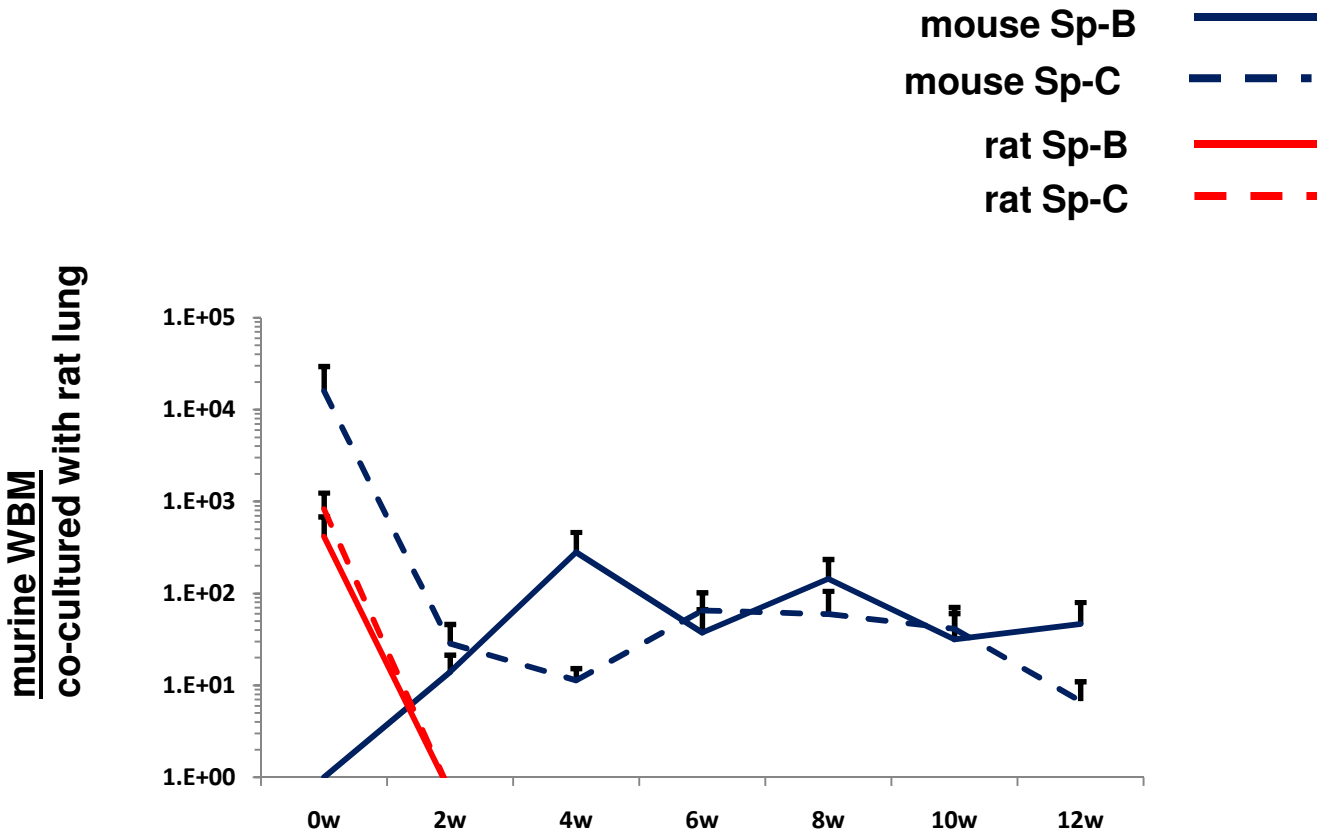
Observation	**Potential Explanation**
Rat-specific Sp-B found in murine WBM	Contamination from rat lung
Murine-specific Sp-B found in murine WBM	Transfer of a transcriptional element

Long-term hybrid co-culture

de novo production of surfactant mRNA

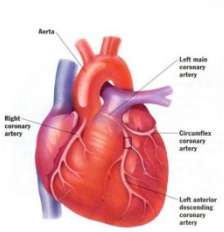


RT-PCR on co-cultured WBM using species-specific primers



The early mRNA induction is Rnase sensitive while the long term “epigenetic”changes are pronase/RNase sensitive.
Thus RNA transfer is responsible for early changes while RNA (Protein protected) or protein transfer may be responsible for long term stable phenotype changes.

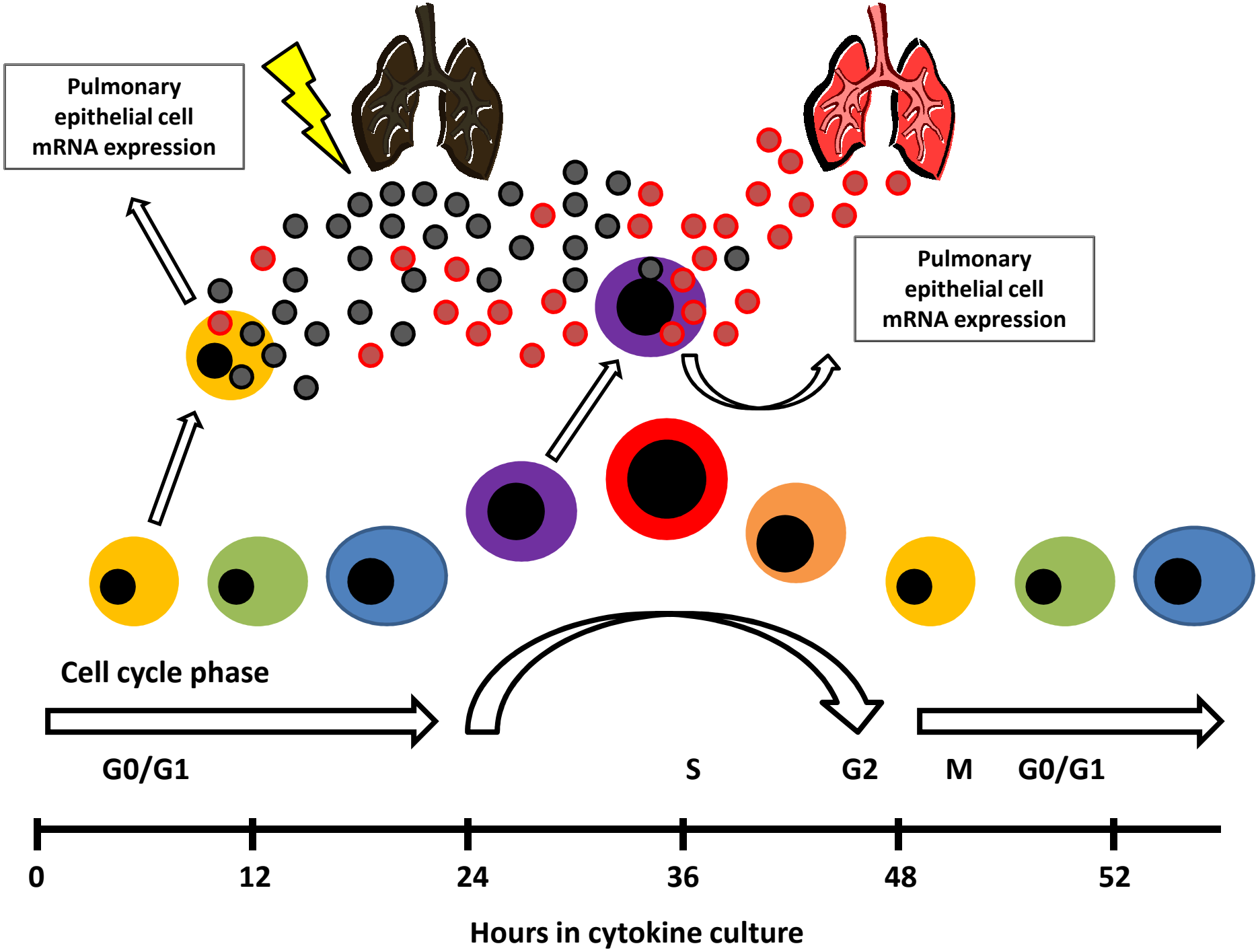
0riginator Microvesicle Target cell Modulated cell

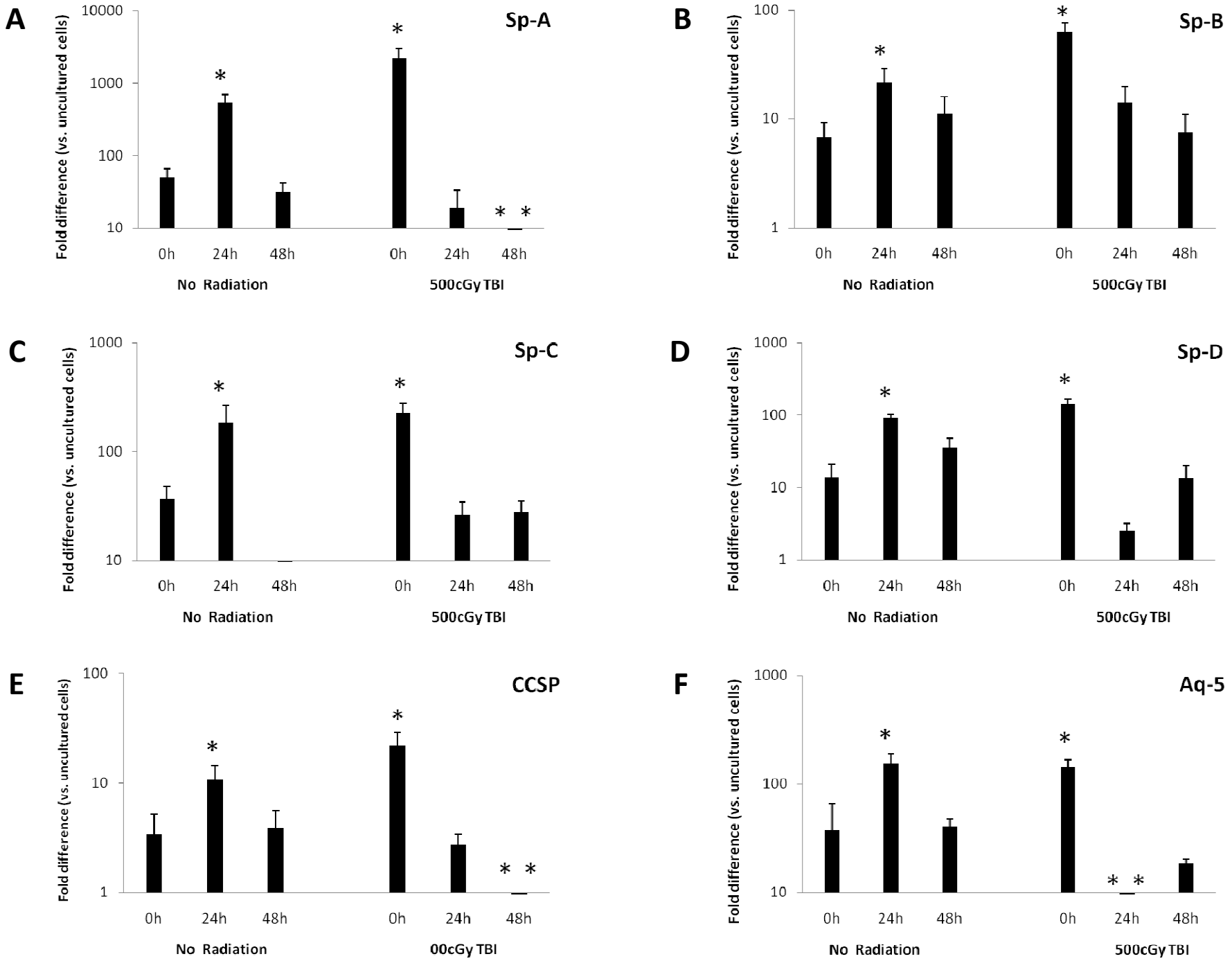


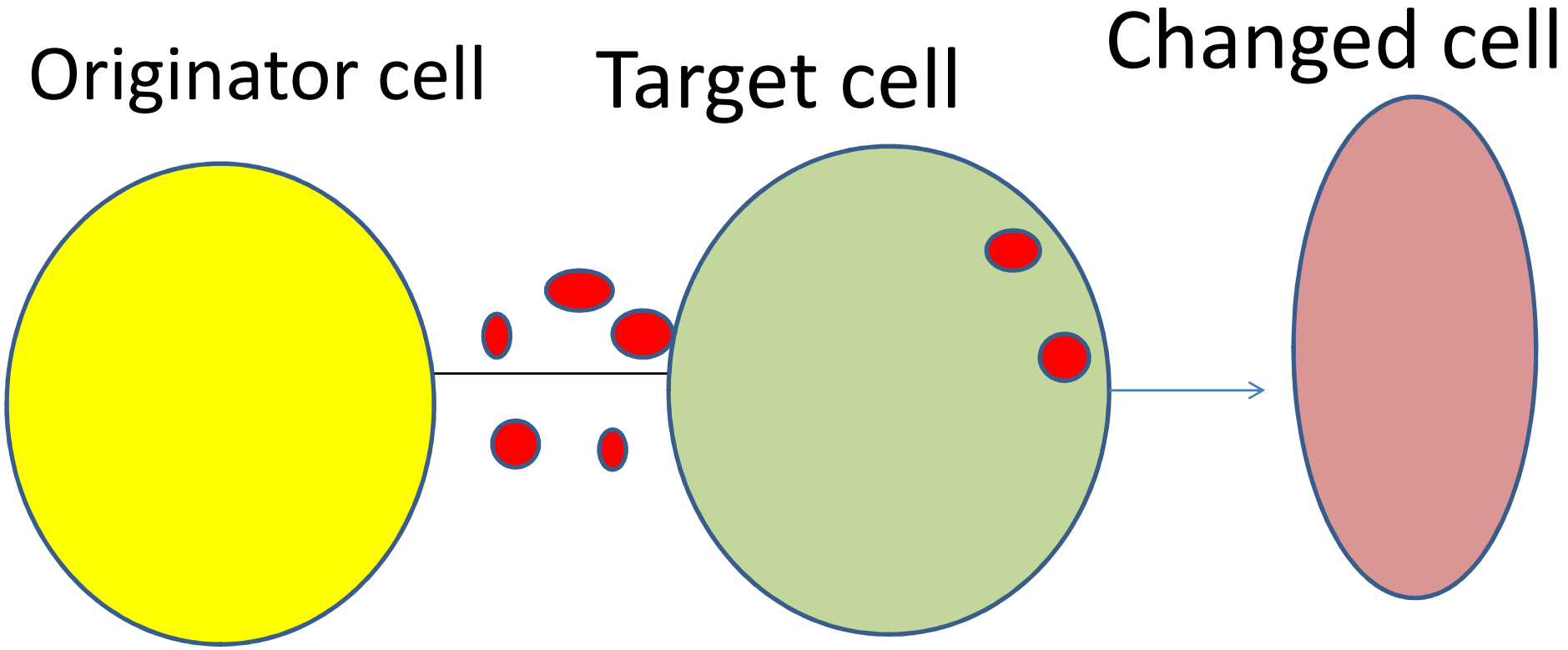







Pulmonary epithelial cell mRNA expression
Pulmonary epithelial cell mRNA expression
Cell cycle phase
G0/G1
S
G2
M
G0/G1
0
12
24
36
48
52
Hours in cytokine culture


A
Sp-A
Fold difference (vs. uncultured cells)
10000
1000
100
10
0h
24h
48h
0h
24h
48h
No Radiation
500cGy TBI
B
Sp-B
100
10
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C
Sp-C
1000
100
10
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Sp-D
E
CCSP
00cGy TBI
F
Aq-5

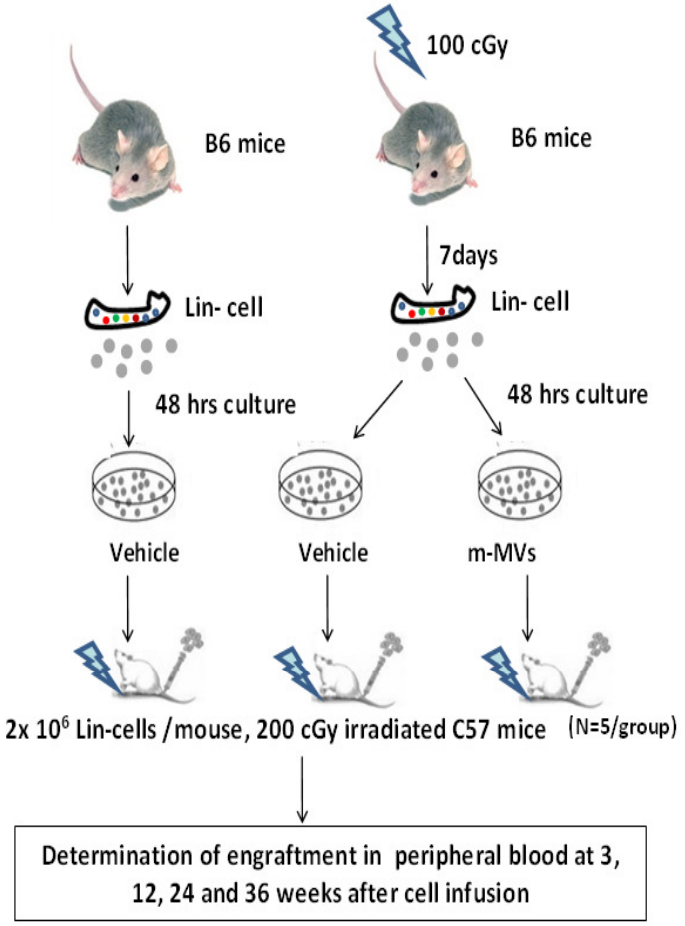


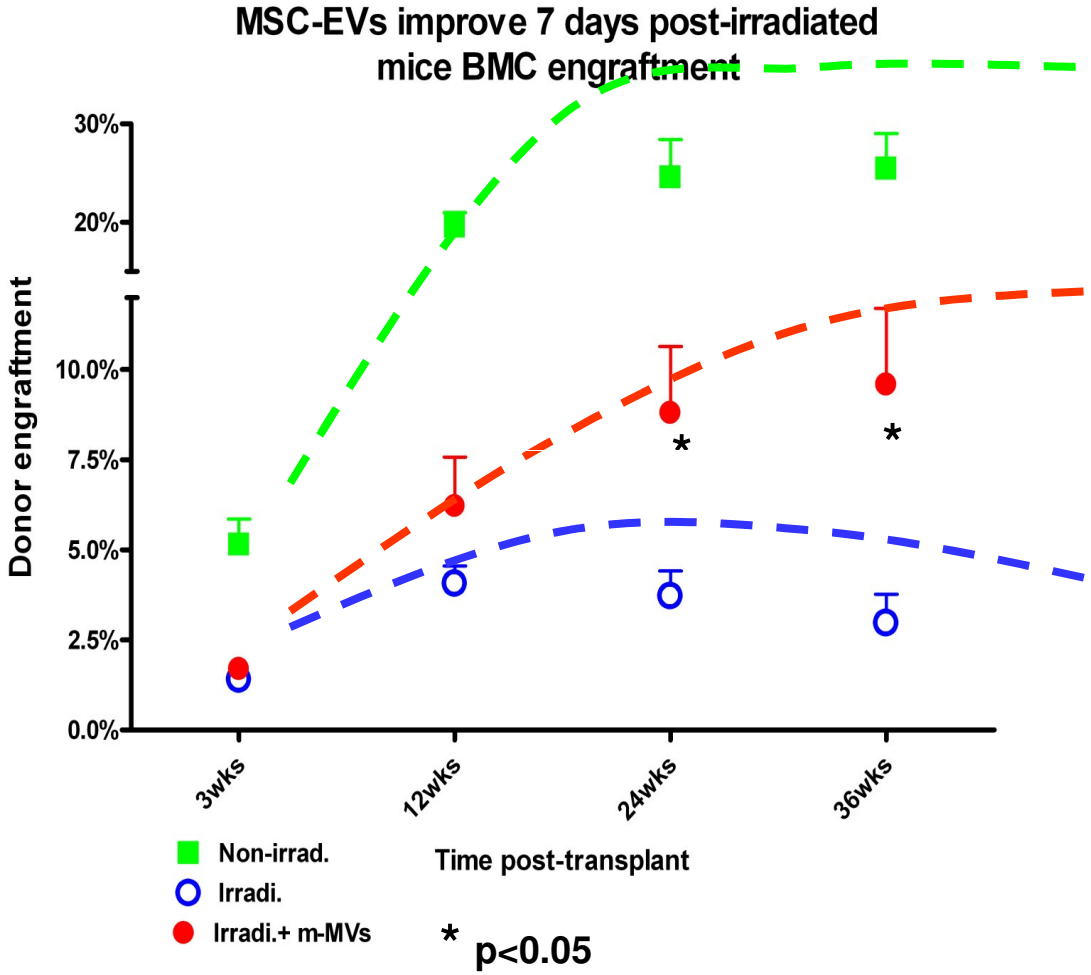
Ipigenetic cell fate
change through vesicles

Vesicles to correct cell injury

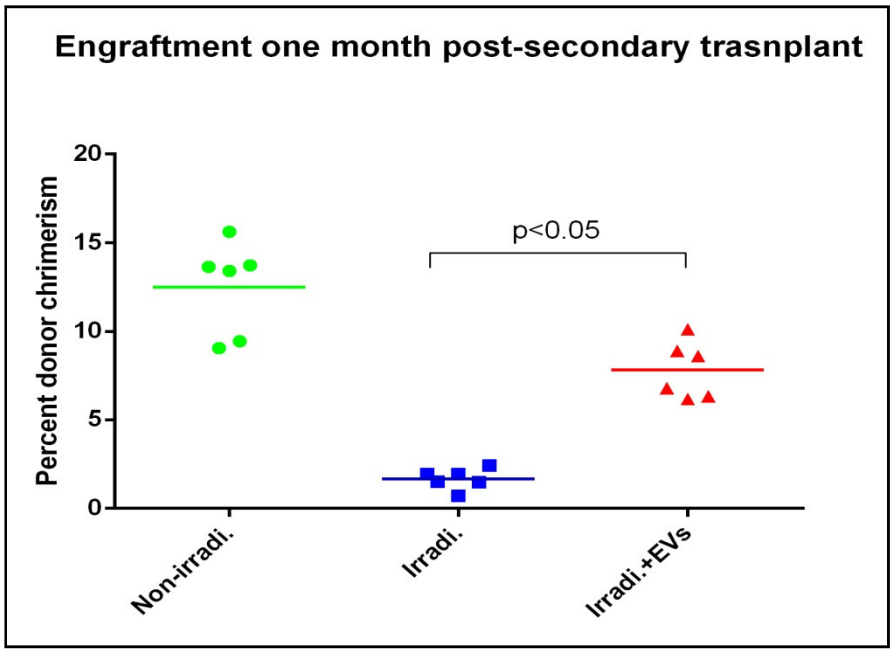
- **Mesenchymal derived vesicles:**
- **To reverse irradiation induced marrow stem cells damage**
- **To reverse renal injury mediated by ischemia-reperfusion or cis platinum**
- **To reverse Tylenol induced liver damage**

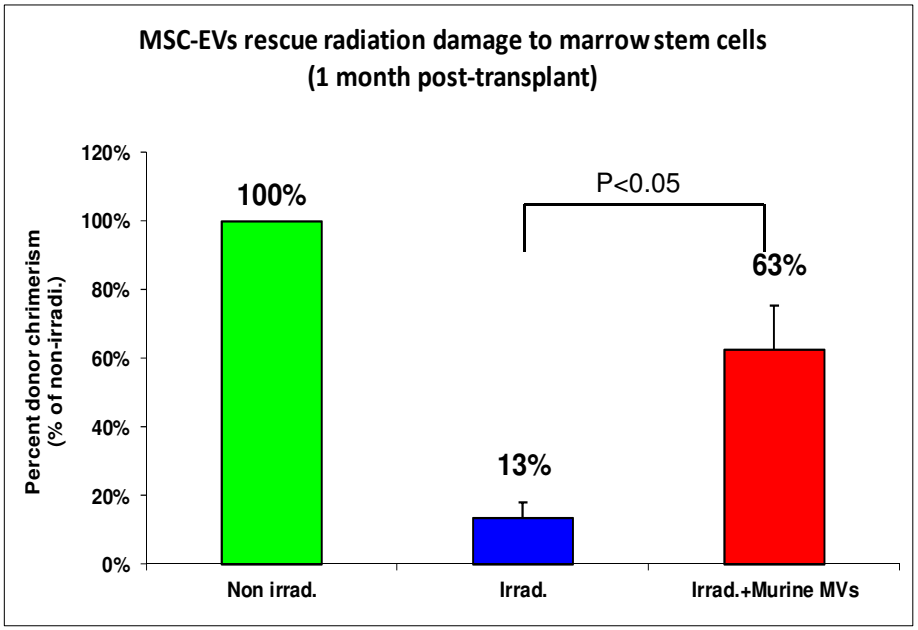
MSC-EVs rescue radiation damage to marrow stem cells

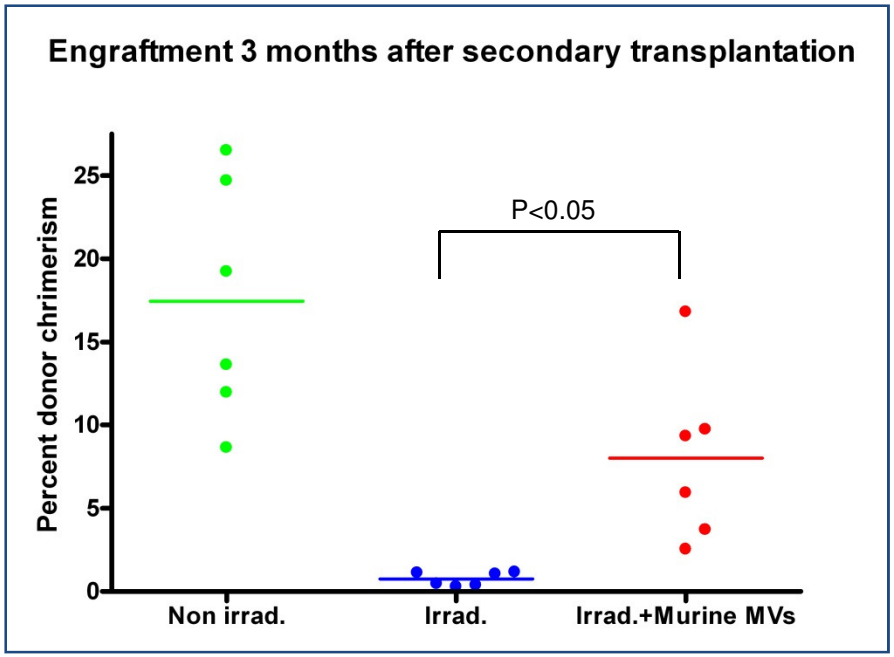


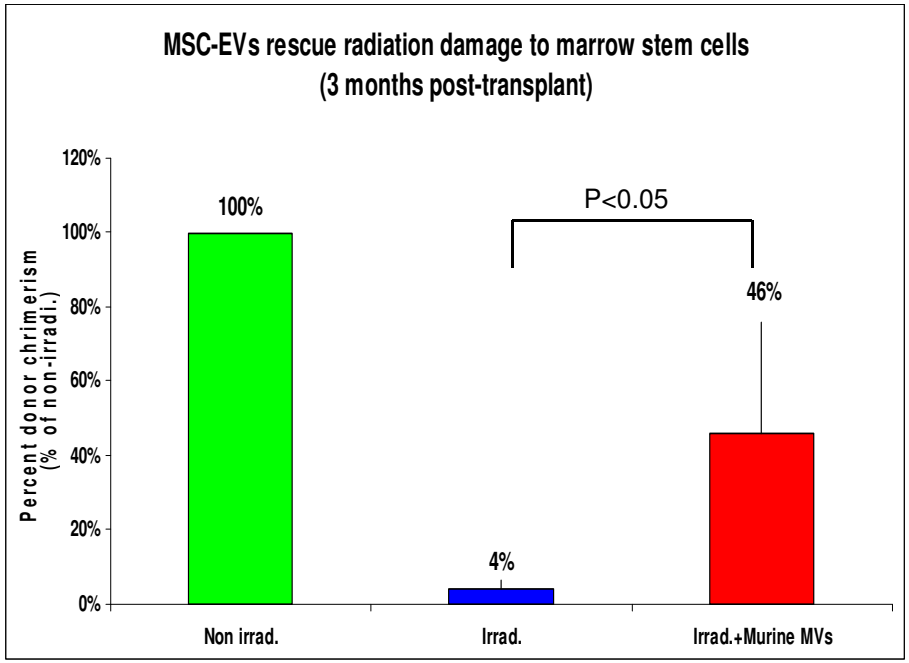


Experiment # 1920

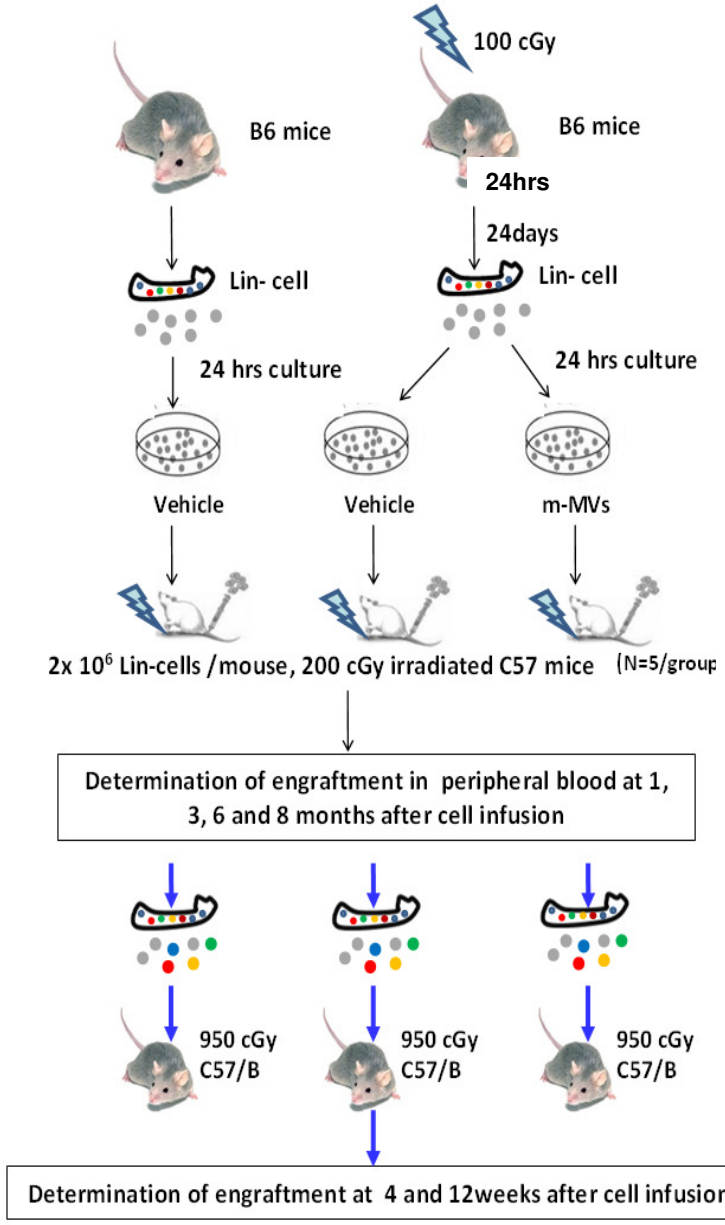


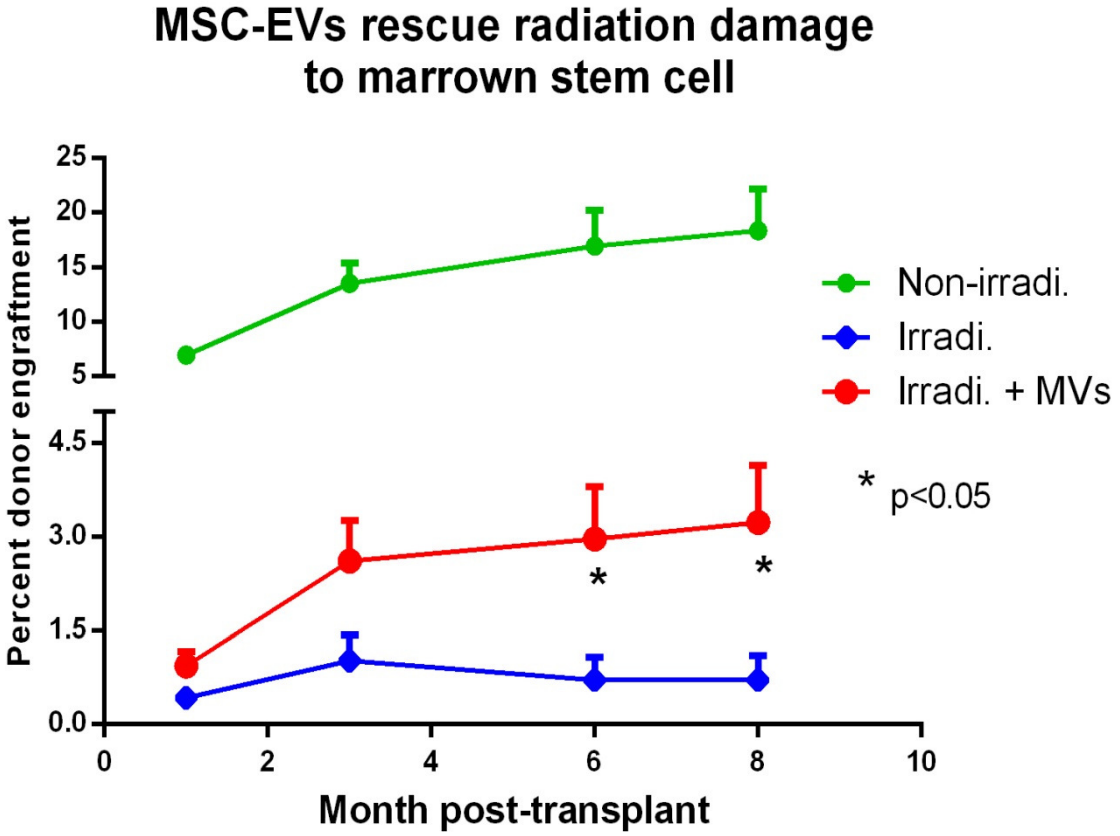




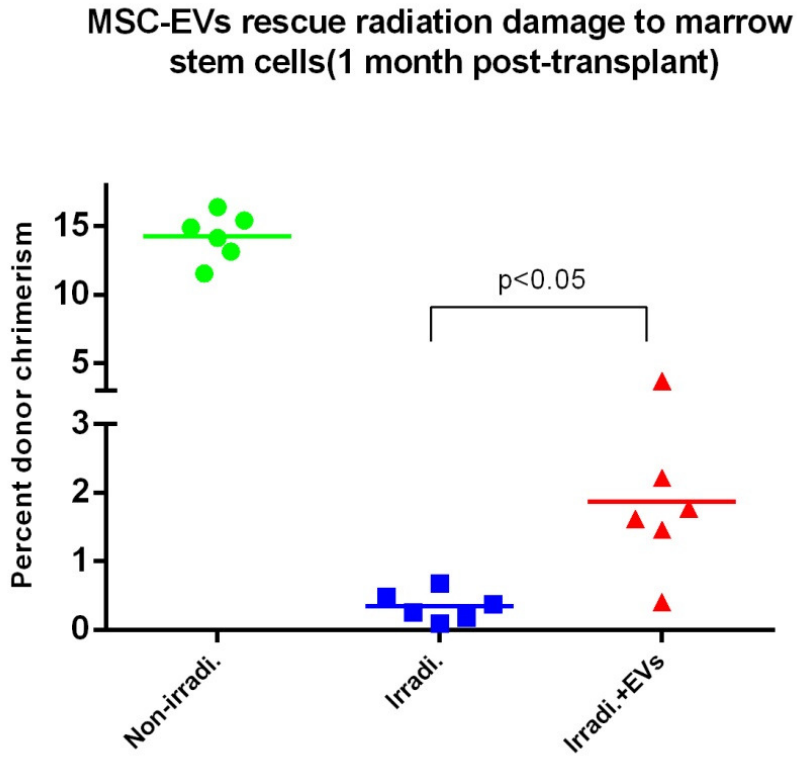


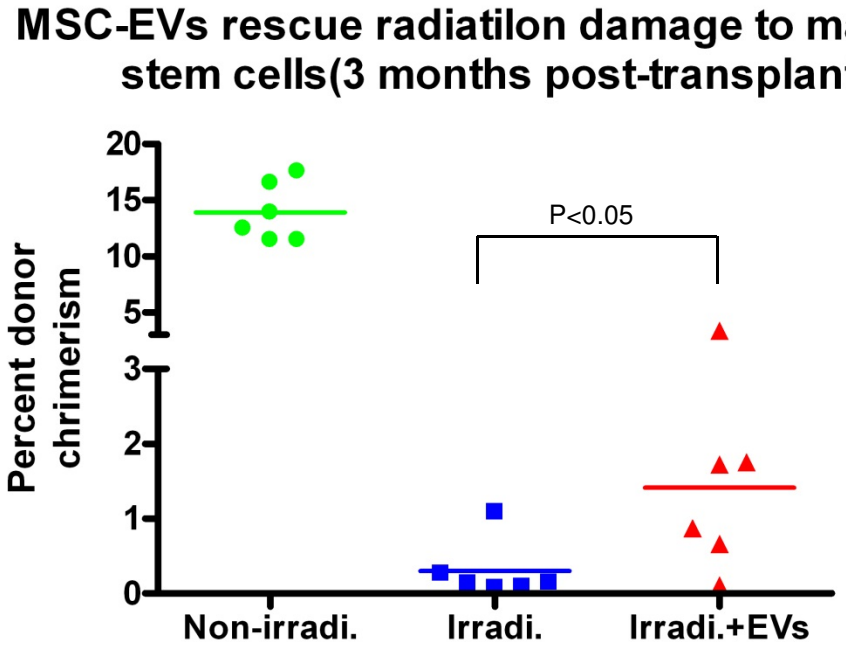
Experiment # 1920A





Experiment # 1932

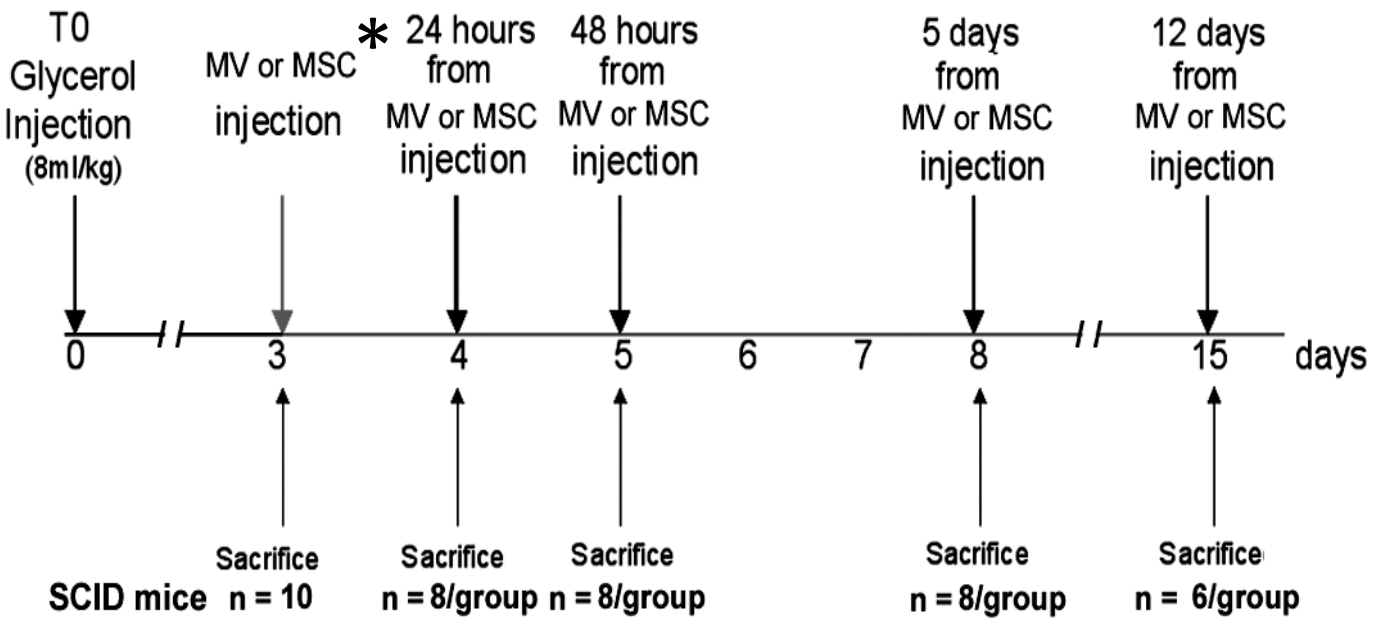




Experiment # 1932A

MVs Protect Against Acute Tubular Injury

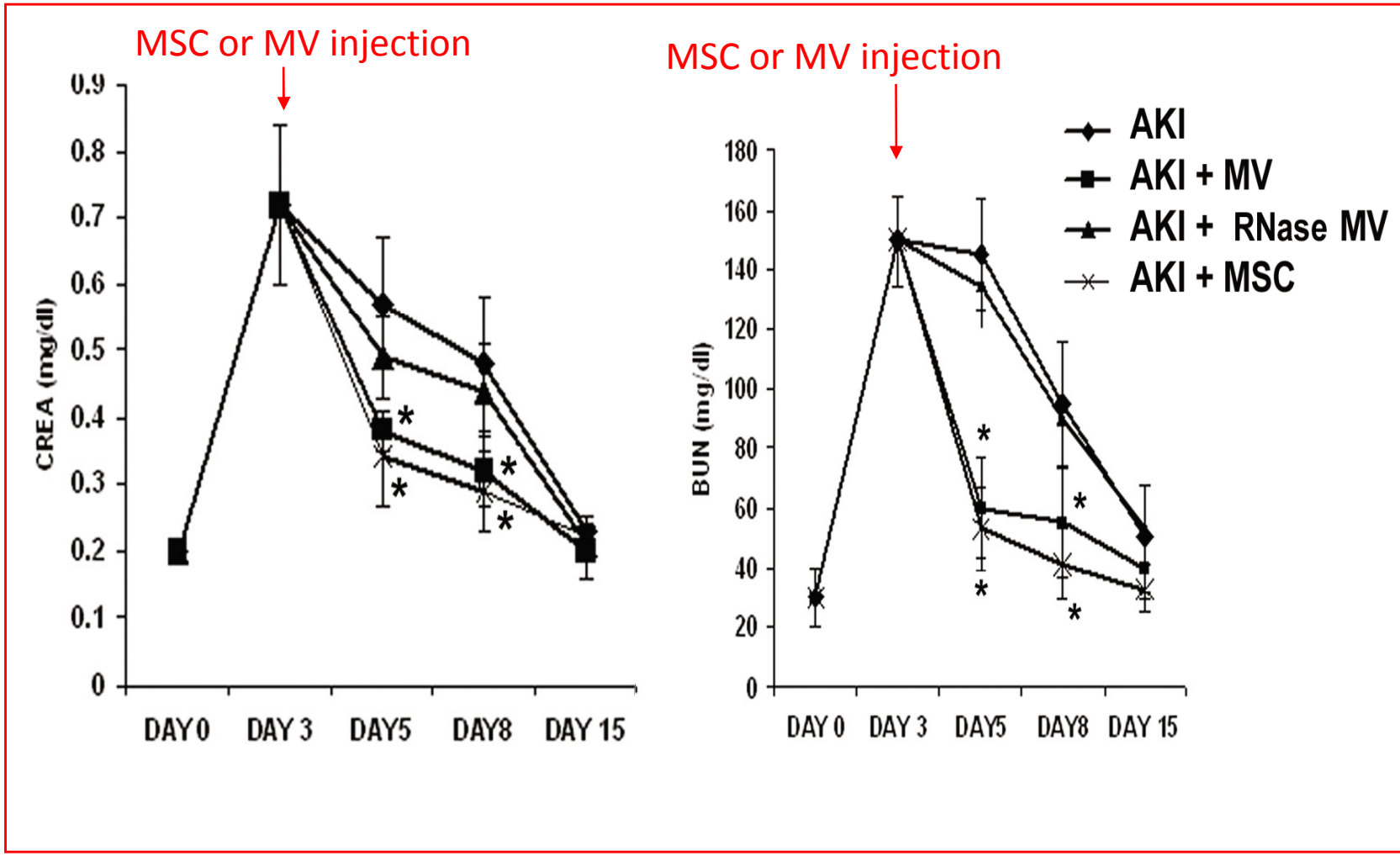
Murine model of Acute Kidney Injury (AKI)



*: 75,000 MSC or 15 µg of MV

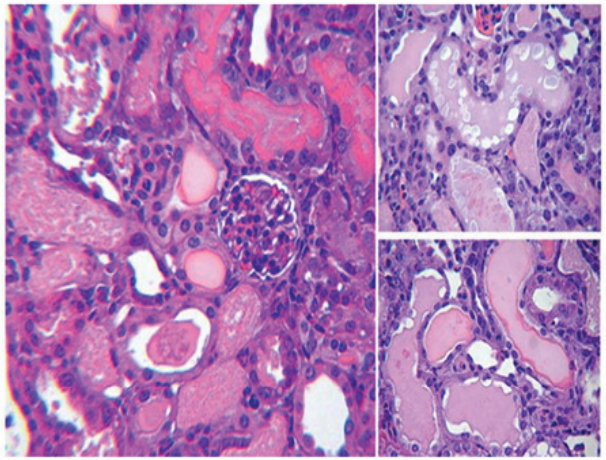
MVs Protect Against Acute Tubular Injury

Functional Recovery

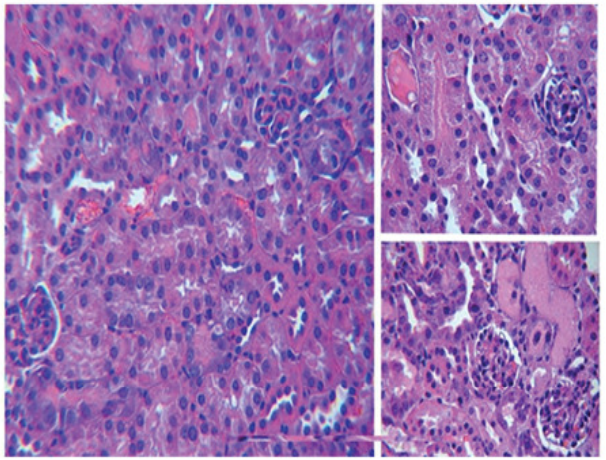


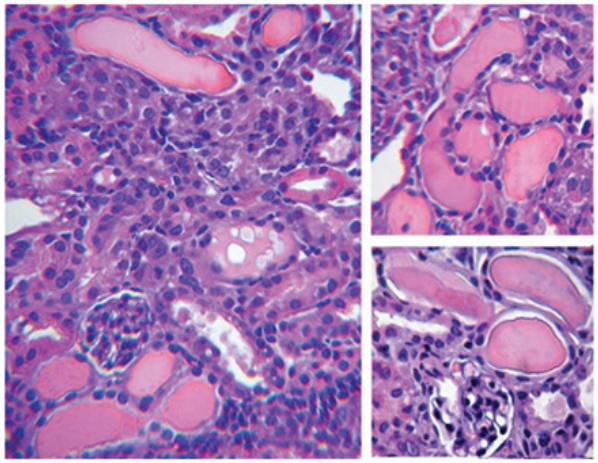
MVs Protect Against Acute Tubular Injury

AKI

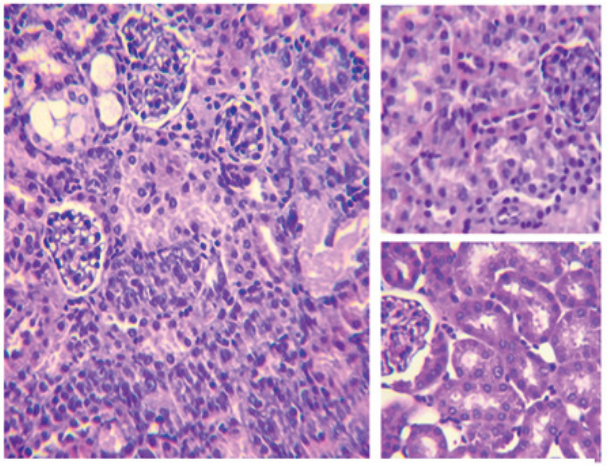


AKI+MV





AKI+RNaseMV



AKI+MSC

Other therapeutic models

- Pulmonary hypertension
- Liver damage
- Prostate cancer

Vesicles as a therapeutic vehicle

- Delivers genetic information inducing stable epigenetic changes.
- Stable for storage at – 20 degree C in 1% DMSO.
- Critical variable – cell source of vesicles and treatment of cell source