8/21/2014

Activation of Growth Factor Complex Leads to Therapeutic Benefits of Laser Photobiomodulation on Stem Cells

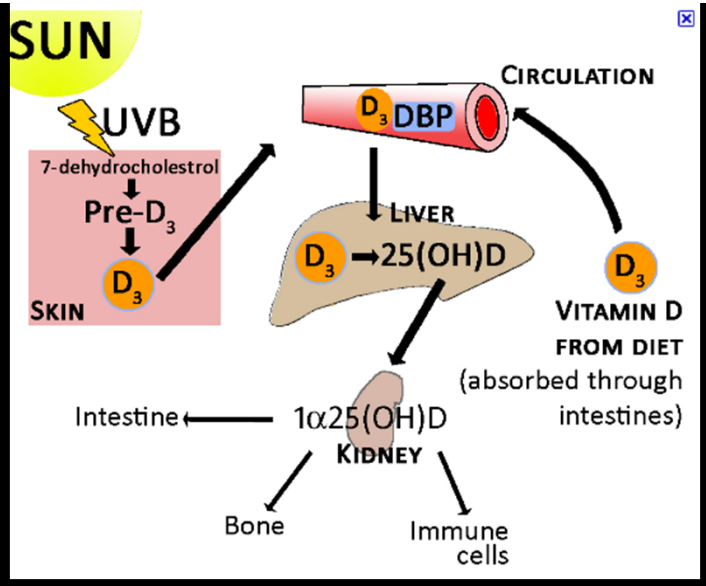
Praveen R Arany DDS, PhD
Assistant Clinical Investigator,
Chief, Cell regulation and Control unit,
NIDCR, NIH, Bethesda MD
praveen.arany@nih.gov

The presenter has no conflicts of interests with the work presented.
This presentation reflects the opinions of the speaker and does not necessarily represent the opinions of the Department of Health and Human services, US government or the National Institutes of Health.

Talk Outline

I. Introduction
II. Research (Molecular mechanism)
III. Applications ☞ Lab
☞ Clinic

Light in Health



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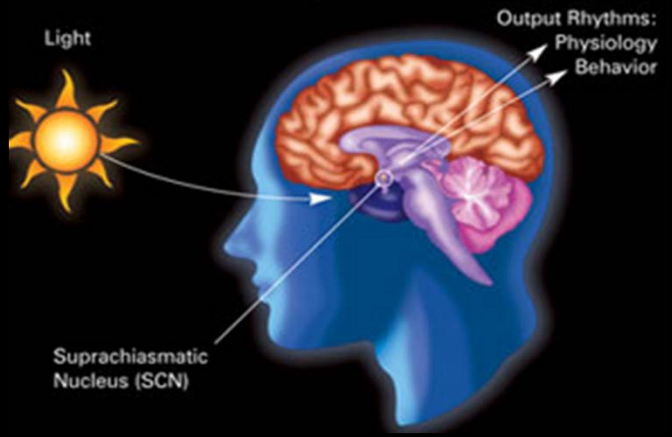
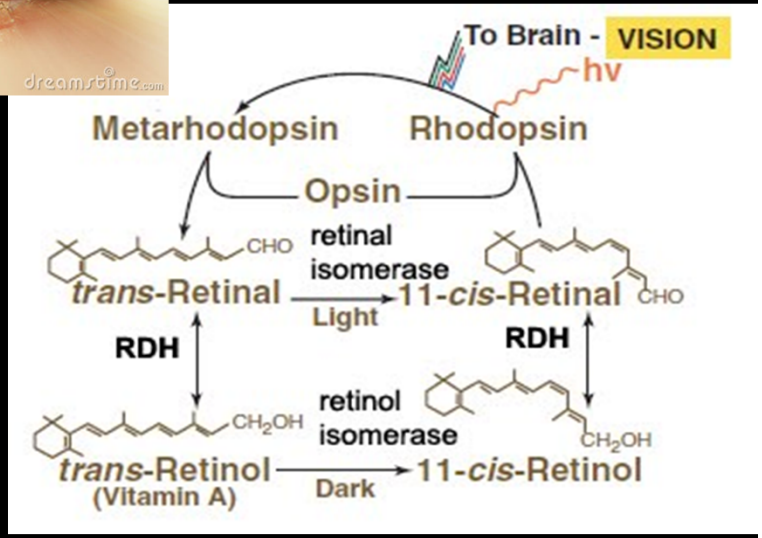
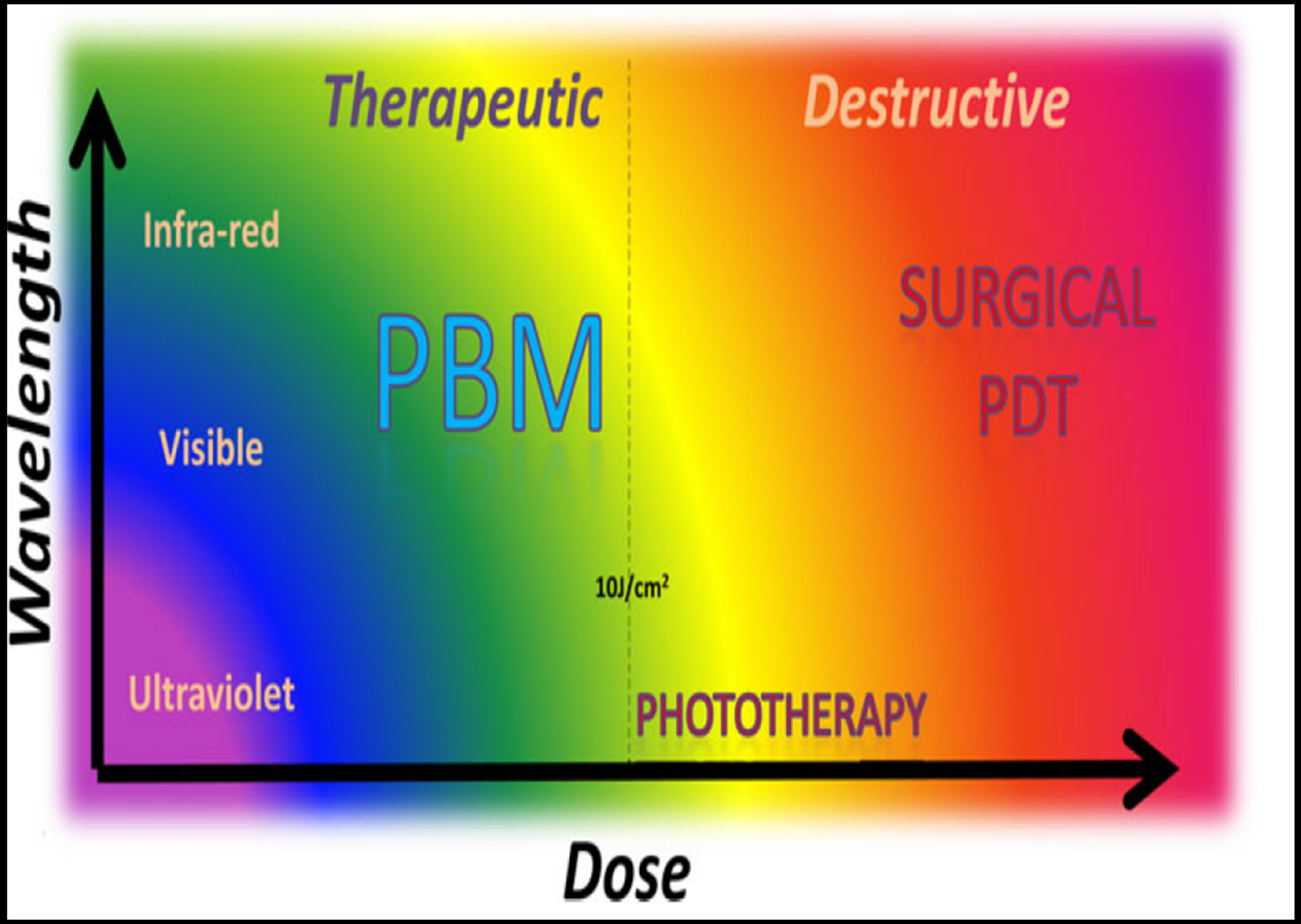




Circadian Rhythm Psychological state



Arany PR **Photmed Las Surg** 2012, 30, 9, 1

Photobiomodulation

"Use of non-ionizing source of photonic energy that generates non-thermal, therapeutic effects."

Inhibit – negative processes

☞ Pain, Inflammation, aberrant immune

Promote – positive processes

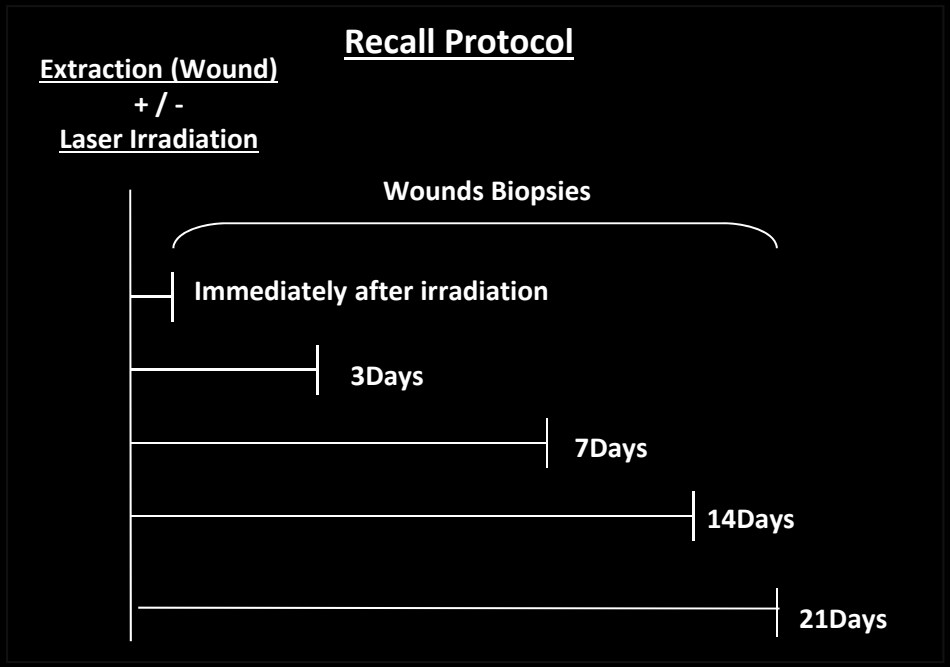
☞ Wound healing, Tissue regeneration, immune system

Oral (Tooth Extraction Socket) Wound Healing

Clinical study recruiting 30 patients undergoing multiple extractions. *Circa* 1999

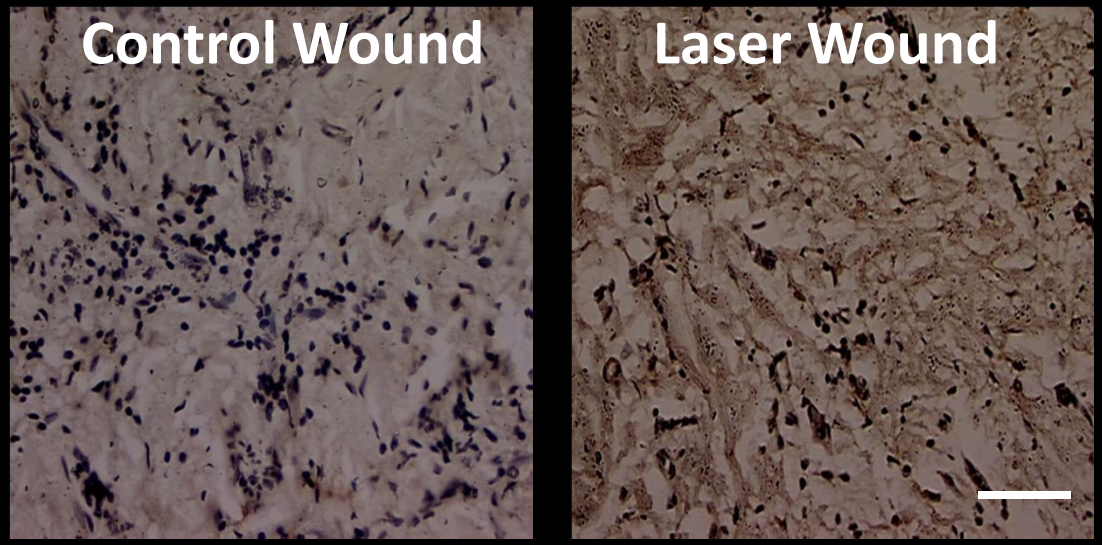
Study Follow up

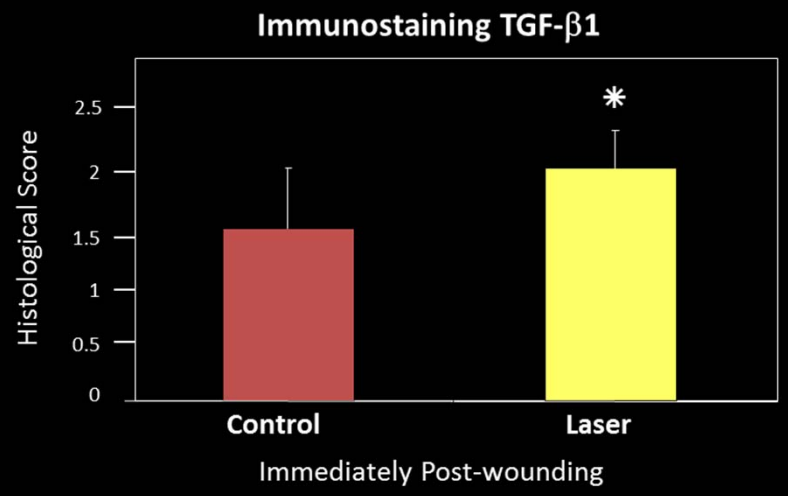
Study Follow up



Same Patient (their own control) healing design critical!!!

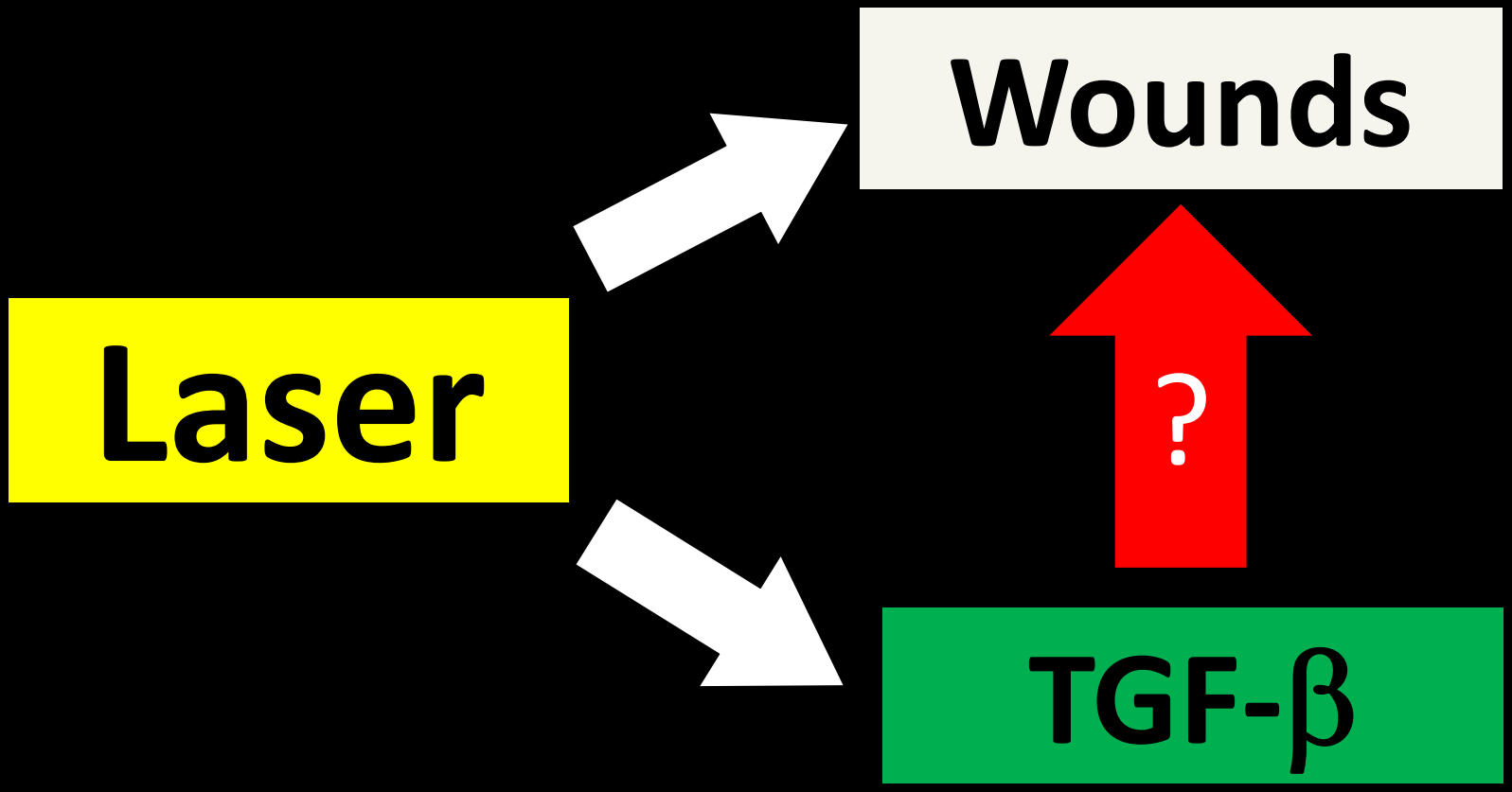
Laser wounds have increased TGF-β1 Expression





Arany PR *et al* **Wound Rep and Regen** 2007,15,866

Clinical observation



Low power laser treatment was noted to improve oral wound healing.
An concomitant increase in TGF-β was noted.

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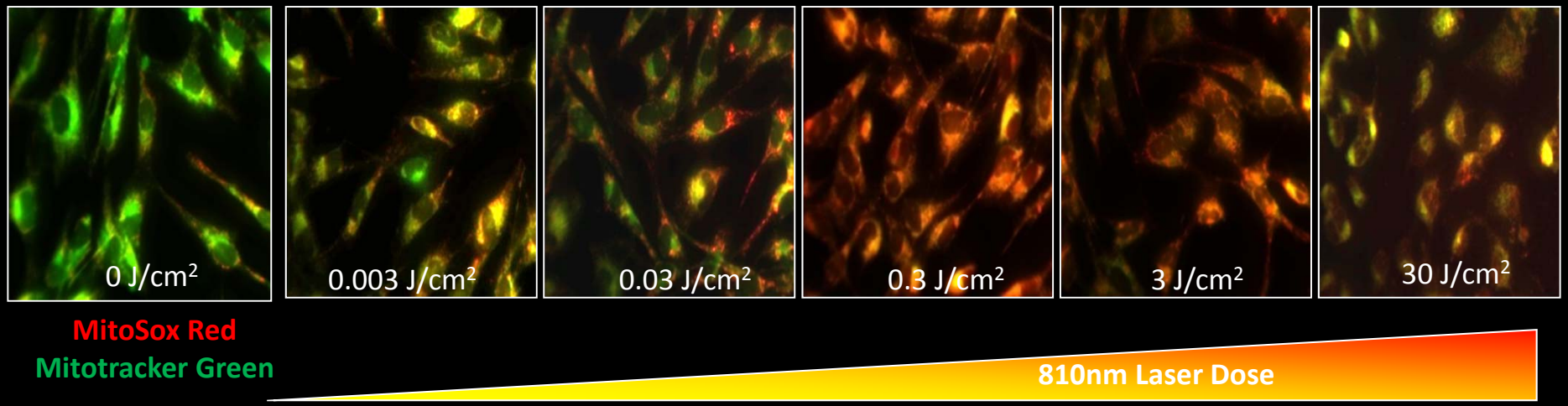
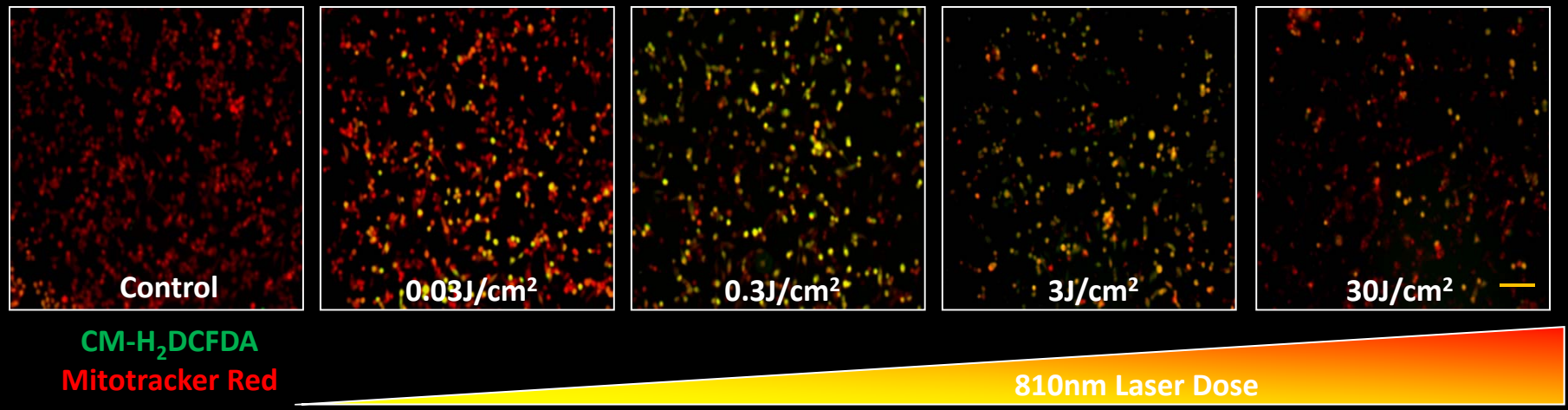
Low power laser induces ROS

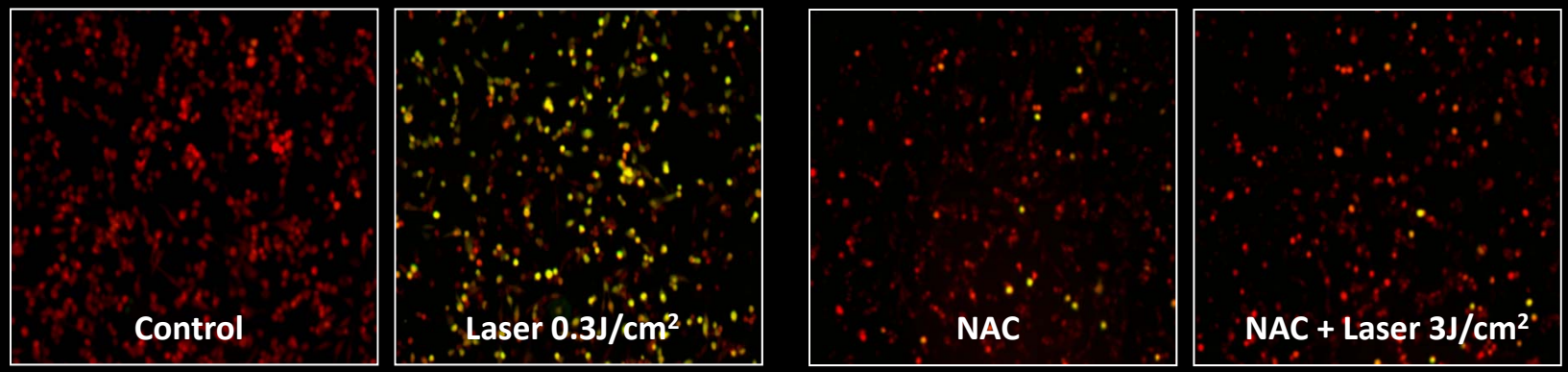


Low power lasers can induce specific ROS namely Superoxide, Hydrogen peroxide and Hydroxyl radical (*not* Nitric oxide)

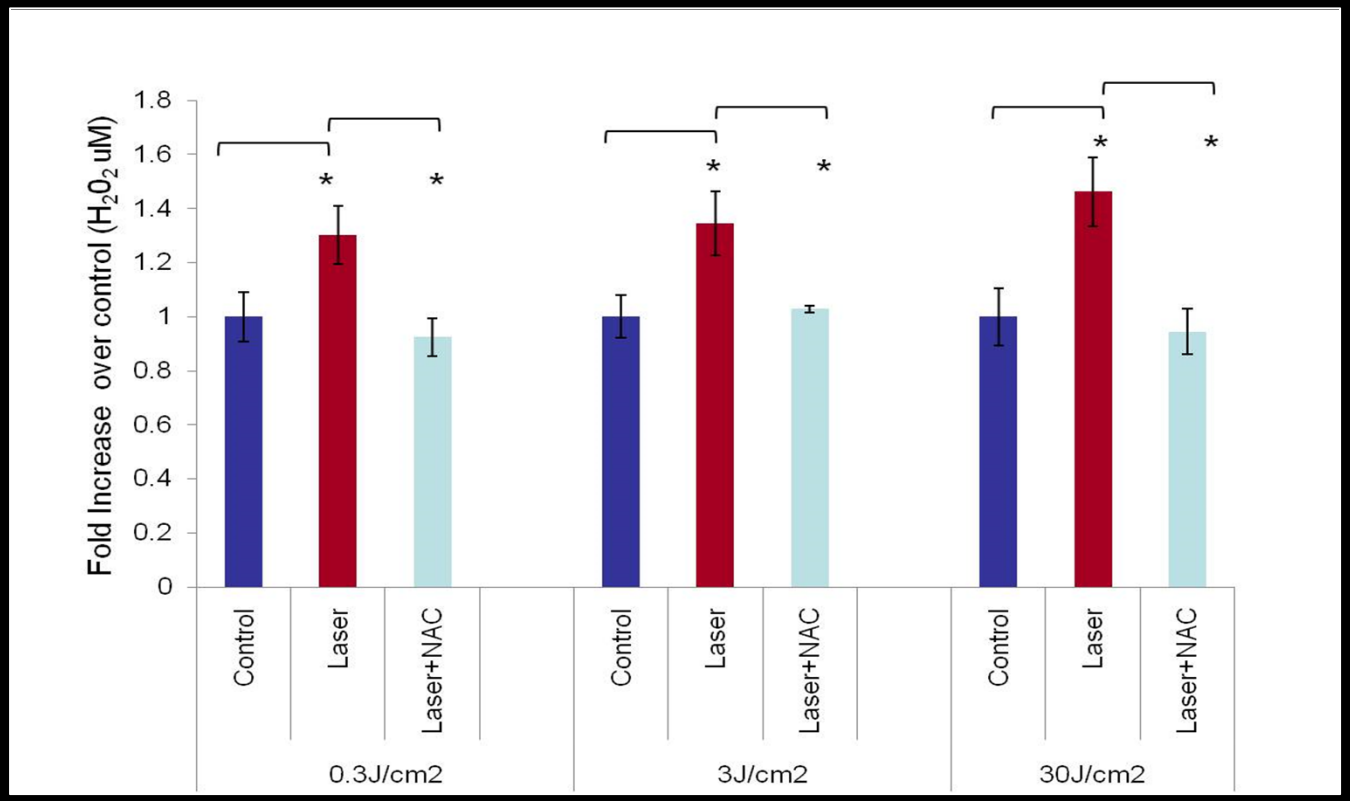
Michael Hamblin, MGH

CM-H_2DCFDA for Hydrogen Peroxide (MvLu1 cells)

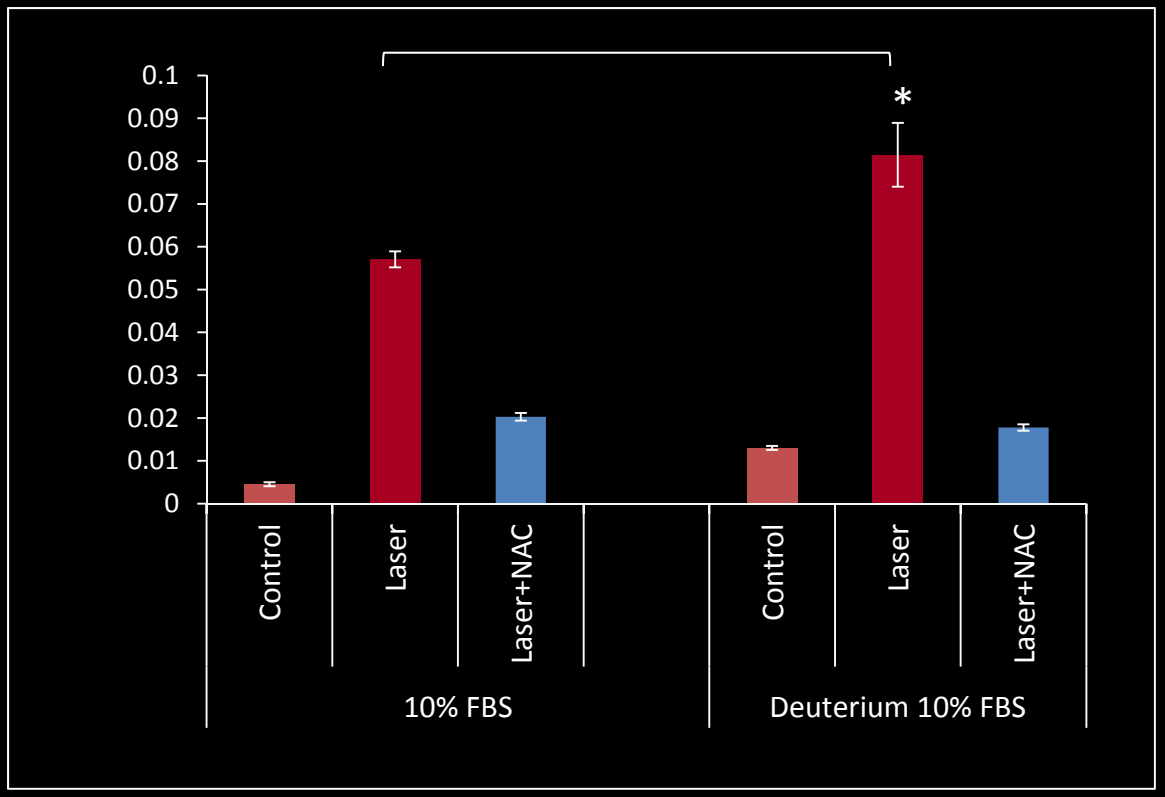




Amplex UltraRed for Hydrogen Peroxide



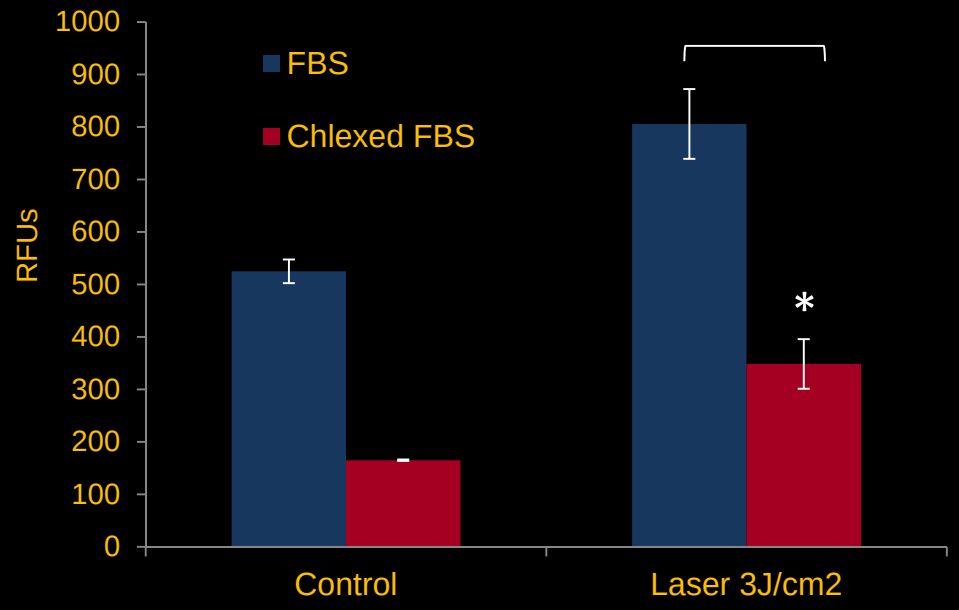
Amplex UltraRed for Hydrogen Peroxide



n=3 p<0.05

Gain of Function:
Deuterium – results in increased ROS generation

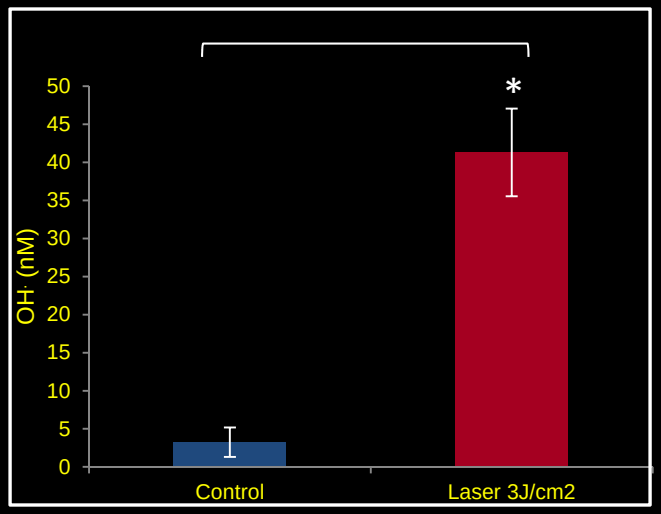
Amplex UltraRed for Hydrogen Peroxide



Loss of Function:
Chelex 100 Resin* – adsorbs metal ions and decreases ROS generation

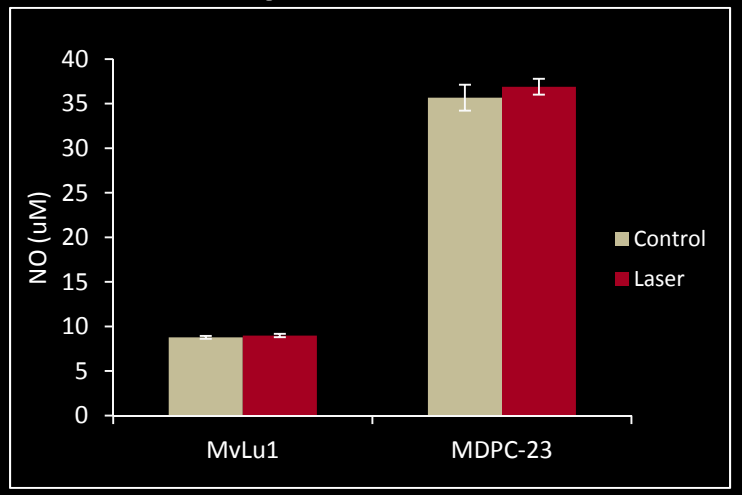
* Styrene-divinylbenzene copolyer with iminodiacetic groups

Proxylfluorescamine for Hydroxyl radical



n=3, p<0.05

Greiss Assay for Nitric Oxide



n=4, p>0.05

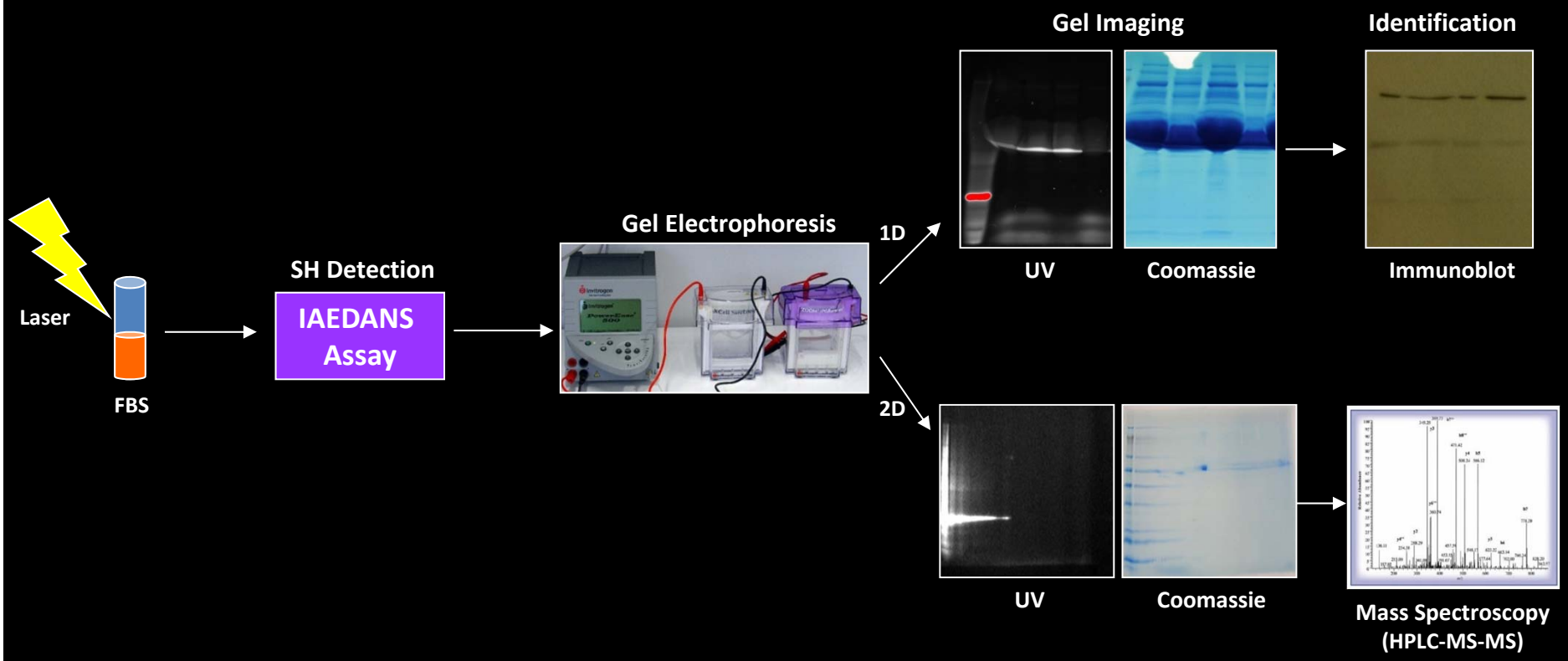
Conclusions

Low power lasers can induce specific reactive oxygen species namely Superoxide, Hydrogen peroxide and Hydroxyl radical

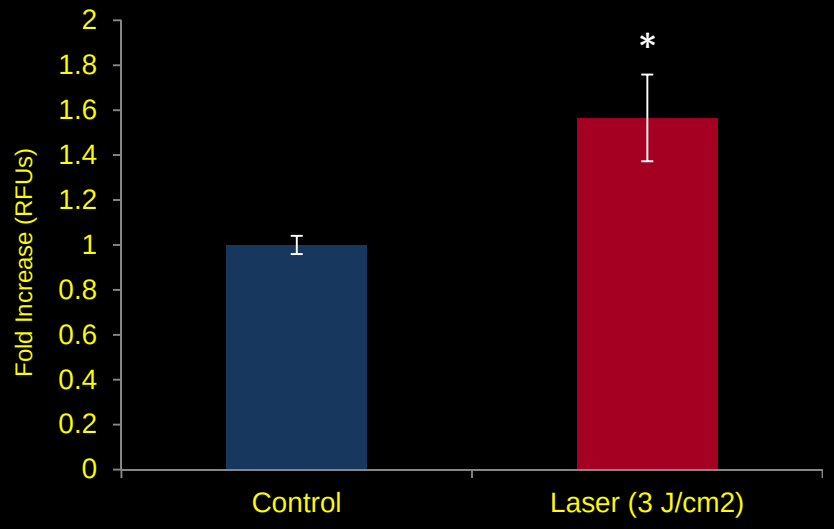
ROS species	Baseline	Laser (3J/cm^2)
Hydroxyl ($OH^{\cdot}$)	3.2 $\pm$ 1 nM	41.3 $\pm$ 5 nM
Superoxide ($O^{\cdot}$)	960.4 $\pm$ 62 nM	1266.1 $\pm$ 163 nM
Hydrogen Peroxide (H_2O_2)	4531.4 $\pm$ 478 nM	57060.8 $\pm$ 1858 nM

Assess ability of LPL generated ROS to activate biological molecules (Latent TGF-β1)

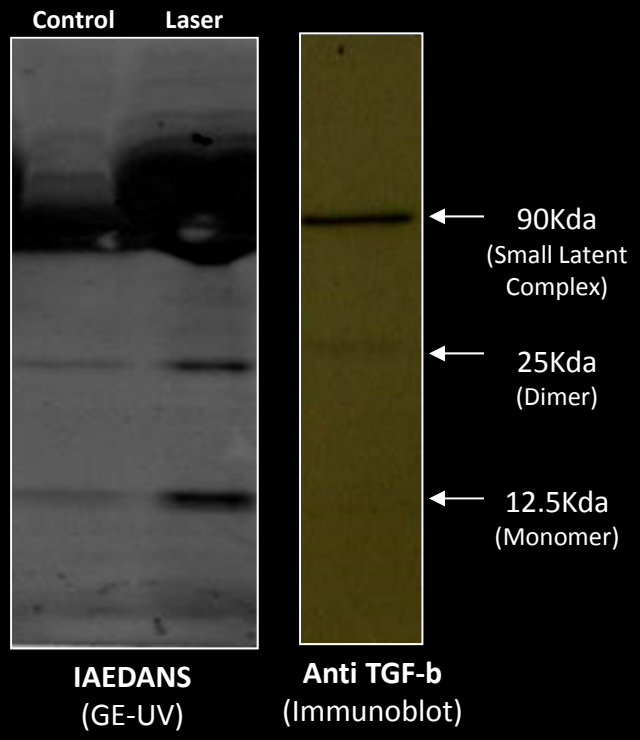
Free Cysteines (Thiols) Screen



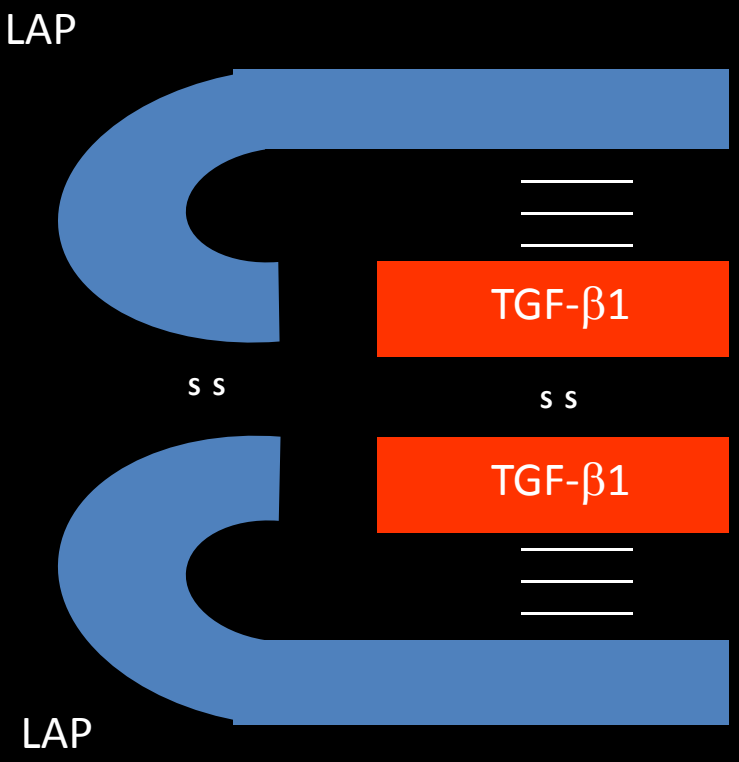
LPL exposes free cysteines in serum complexes, TGF-β is one among them



P<0.05



Activation of Latent TGF-β1



Physico-chemical Modes

- Heat
- Extreme pH
- Proteases
- Radiation

Biologically Active

Generation of a ROS insensitive LTGF-β1

```
beta 1  LSTCKTIDMELVKRKRIEAIRGQILSKLRLASPPSQGEVPPGPLPEAVLALYNSTRDRVA
beta 2  LSTCSTLDMDQFMRKRIEAIRGQILSKLKLTSPPEDYPE-PEEVPPEVISIYNSTRDLLQ
beta 3  LSTCTTLDFGHIKKKRVEAIRGQILSKLRLTSPPE--PTVMTHVPYQVLALYNSTRELLE

                                (112)                (132)
beta 1  GESAEPEP-----EPEADYYAKEVTRVLMVE---THNEIYDKFKQSTHSIYMFFNTSELR
beta 2  EKASRRAAACERERSDEEYYAKEVYKIDMPPFFPSENAIPPTFYRPYFRIVR-FDVSAME
beta 3  EMHGEREEGCTQENTESEYYAKEIHKFDMIQGLAEHNELAVCPKGITSKVFR-FNVSSVE

beta 1  EAVPEPVLLSRAELRLLRLK----LKVEQHVELYQKYSNN-----SWRYLSNRLLAPSDS
beta 2  KNASN---LVKAEFRVFRLQNPKARVPEQRIELYQILKSKDLTSPTQRYIDSKVVKTRAE
beta 3  KNRTN---LFRAEFRVLRVPNPSSKRNEQRIELFQILRPDEHIA-KQRYIGGKNLPTRGT

beta 1  PEWLSFDVTGVVRQWLSRGGEIEGFRLSAHCSC----------DSRDNTLQVD---ING
beta 2  GEWLSFDVTDAVHEWLHHKDRNLGFKISLHCPCCTFVPSNNYIIPNKSEELEARFAGIDG
beta 3  AEWLSFDVTDTVREWLLRRESNLGLEISIHCPCHTFQP-NGDILENIHEVMEIKFKGVDN

                (253)
beta 1  FTTGRRGDLATIH-----GMNRPFLLLMATPLERAQLQ---SSRHRR
beta 2  TSTYTSGDQKTIKSTRKKNSGKTPHLLLMLLPSYRLESQ-QTNRRKKR
beta 3  EDDHGRGDLGRLK---KQKDHHNPHLILMMIPPHRLDNPGQGGQRKKR
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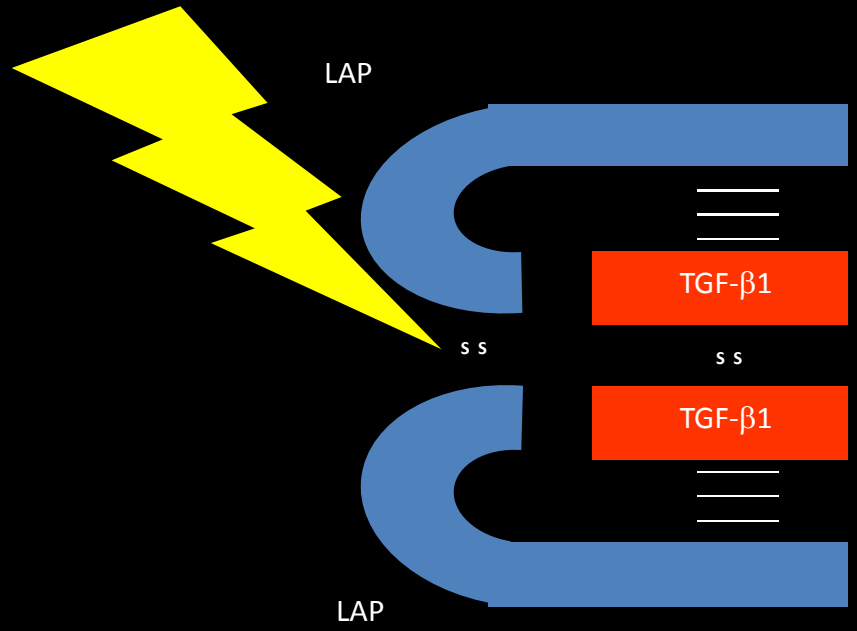
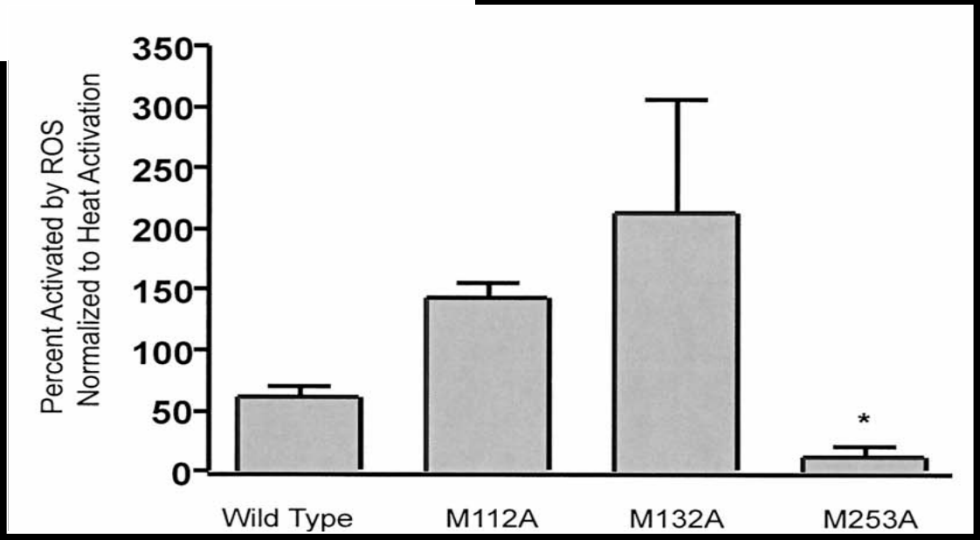


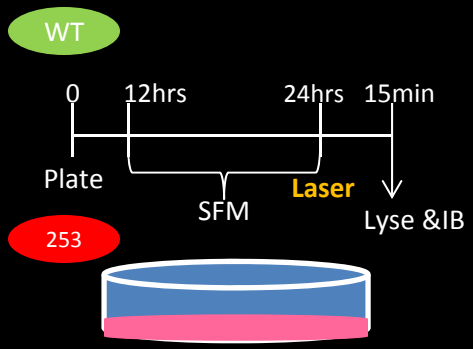


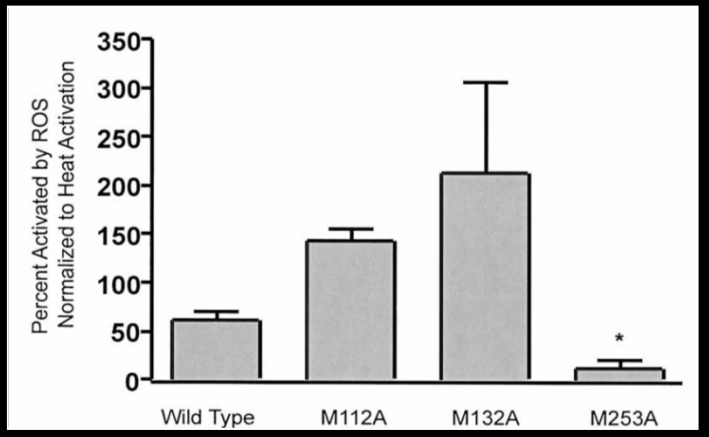
Jobling MF *et al* Rad Res 2008, 166, 839

Mary-Helen Barcellos Hoff, NYUMC

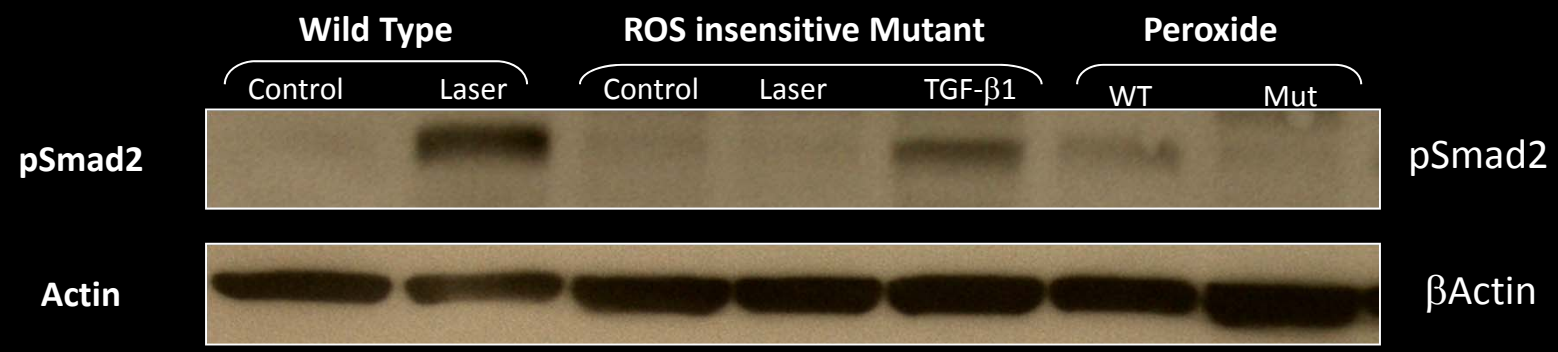
NYU Langone Medical Center

Laser mediates TGF-β activation via ROS





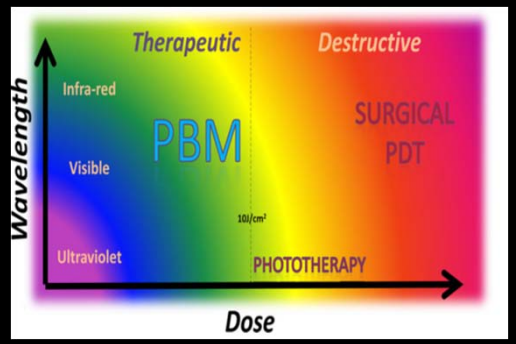
Jobling MF *et al* Rad Res 2008, 166, 839

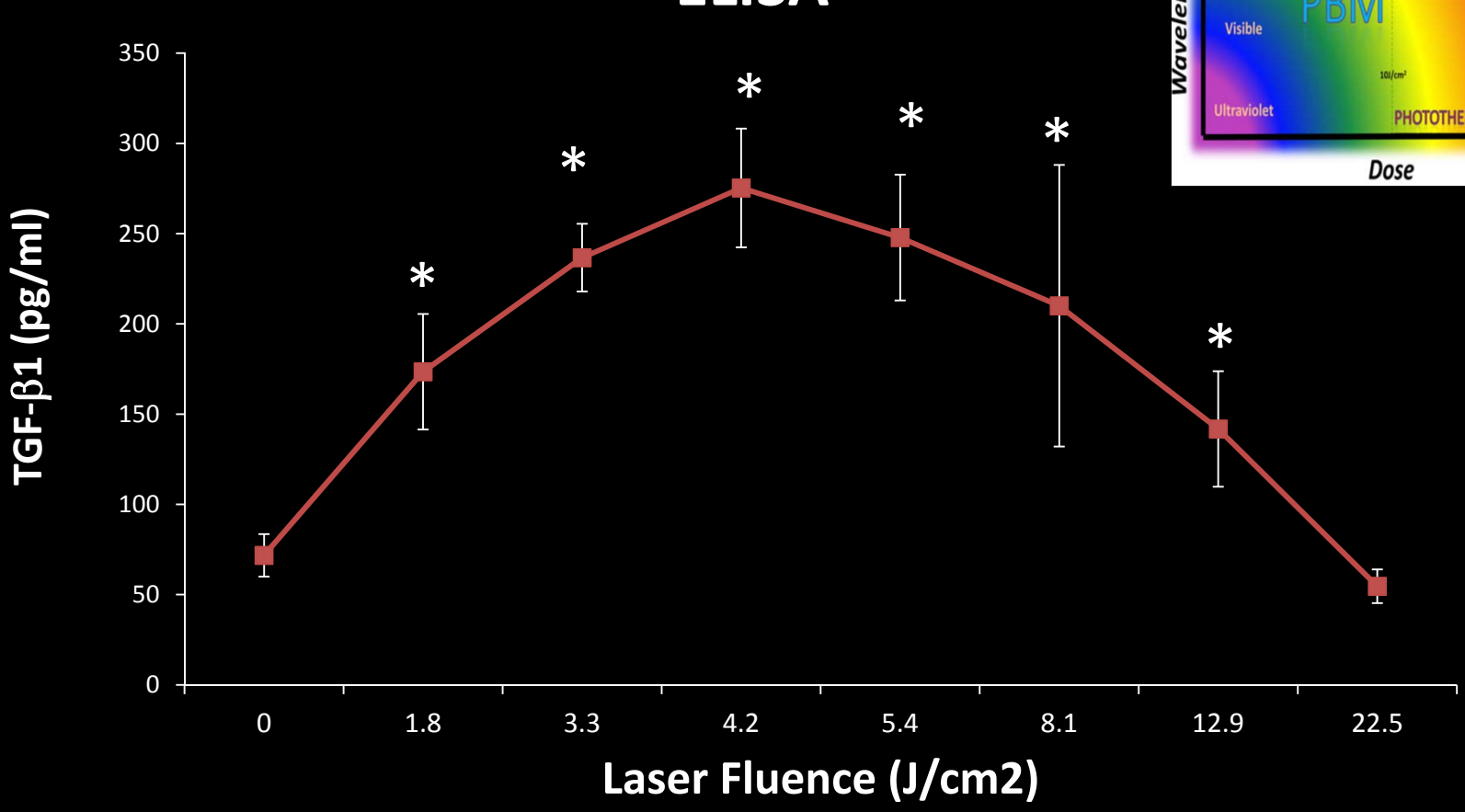


Mary-Helen Barcellos Hoff, NYUMC

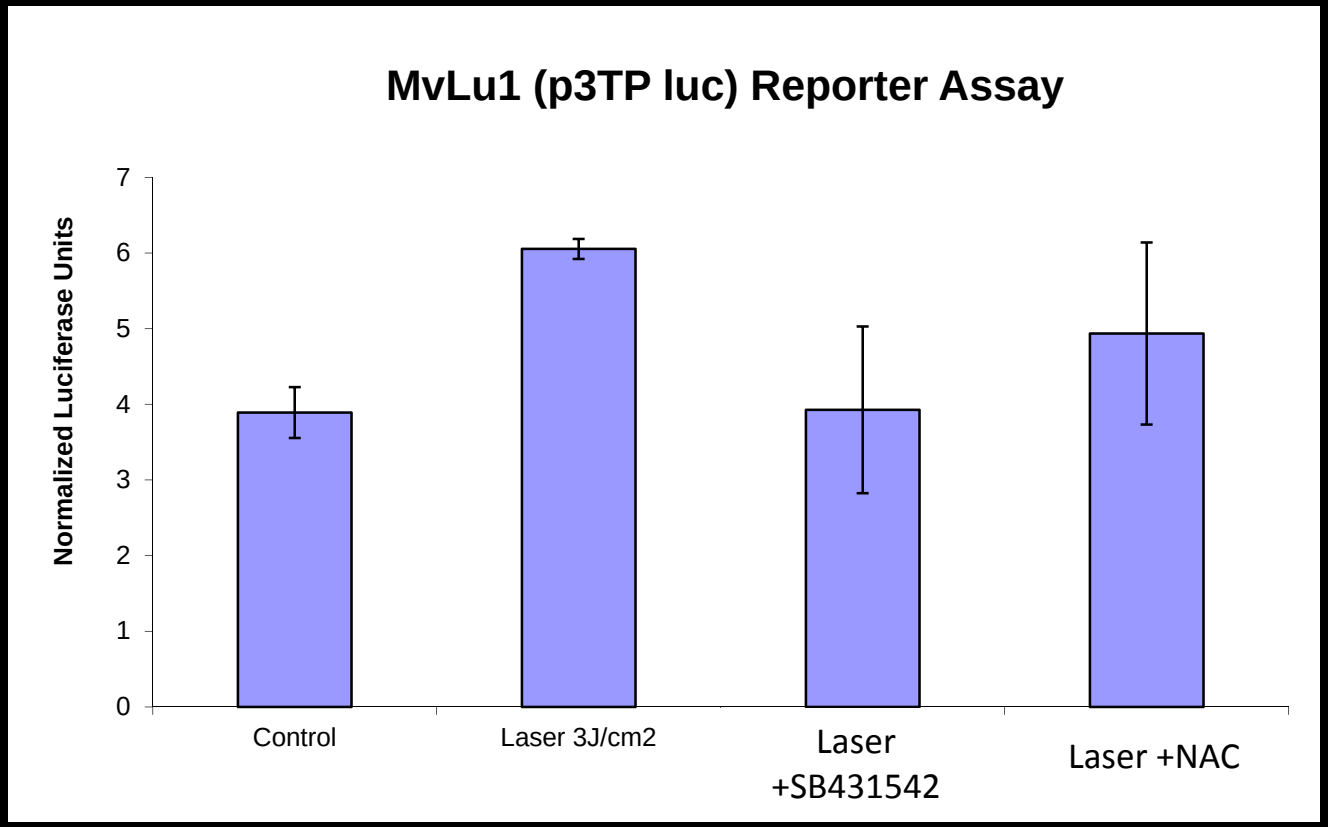
NYU Langone Medical Center

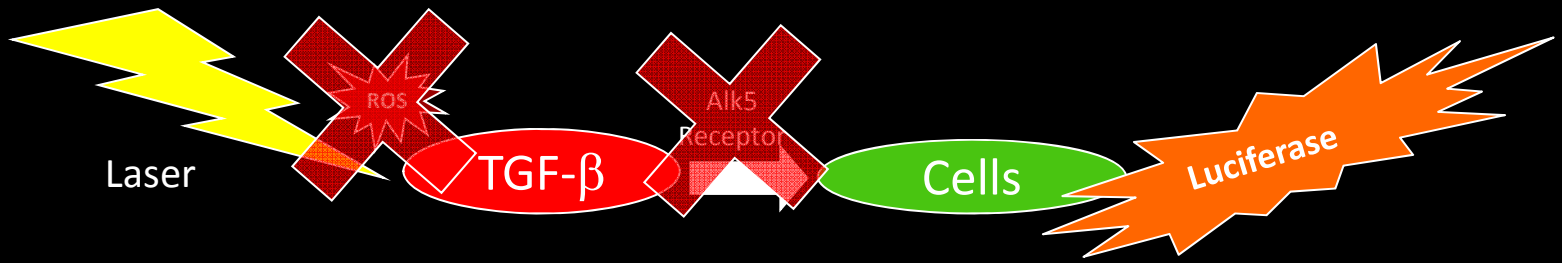
LPL activates Latent TGF-β1





LPL activated TGF-β1 is biologically potent





Dan Rifkin

NYU Langone Medical Center

Conclusions

Low power lasers generate ROS that can, in turn, activate Latent TGF-β1 via a redox sensitive methionine.

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I. Introduction
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III. Applications ☞ Lab
☞ Clinical

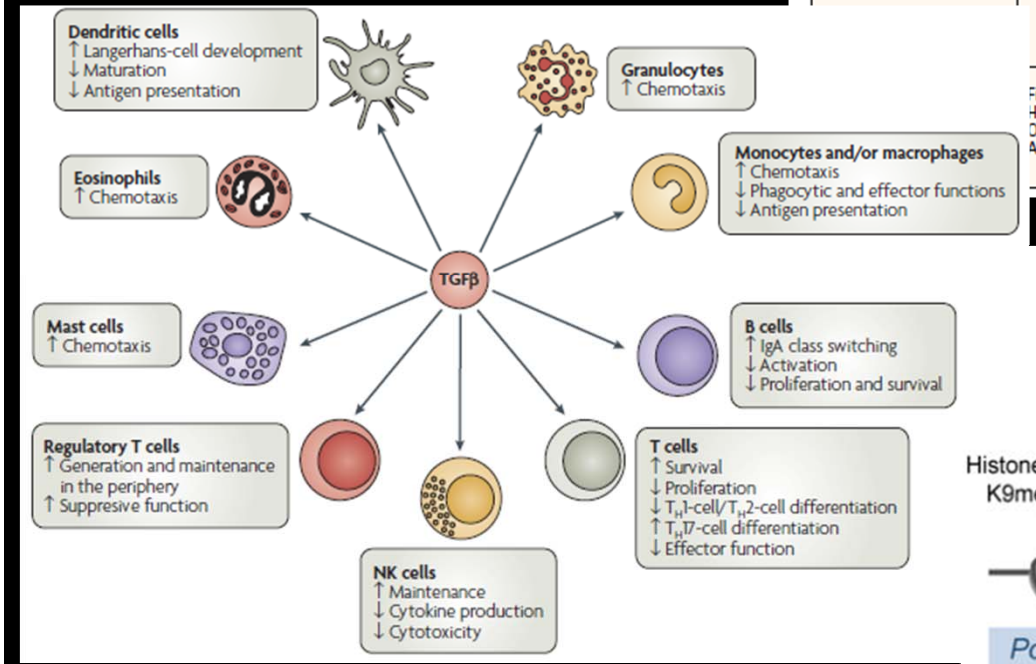
Applications: LPL activated TGF-β1?

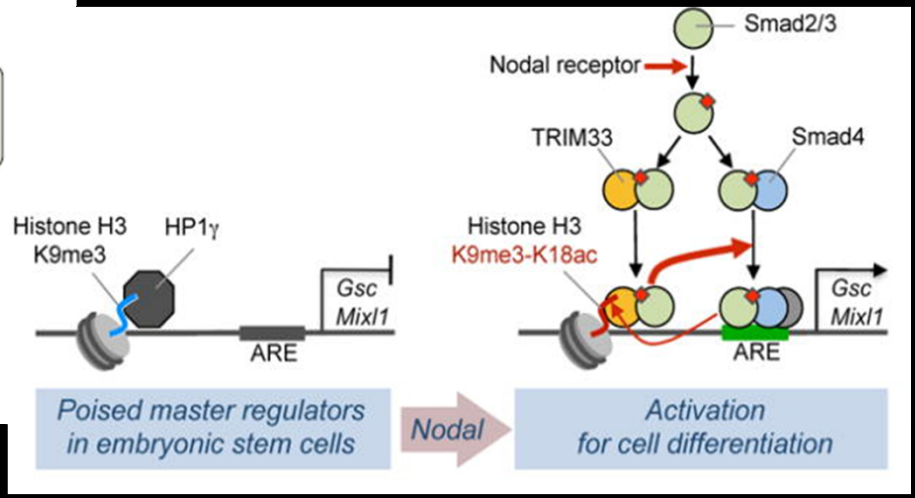
Role of Transforming Growth Factor β in Human Disease

Gerard C. Blobe, M.D., Ph.D.,
William P. Schiemann, Ph.D.,
and Harvey F. Lodish, Ph.D.

TGF-β signaling component	TGF-β	Endoglin	Type II receptors	Type I receptors	Smad2	Smad4
Cancers (somatic mutations)	Increased expression leads to enhanced invasion and metastasis		Colorectal (30%) Gastric (15%) Endometrial Prostate Breast Lung Hepatic Pancreatic Cervical Glioma Head and neck	Breast (16%) Pancreatic Biliary Cervical Chronic lymphocytic leukemia	Colorectal (11%) Lung (7%) Hepatocellular	Pancreatic (50%) Colorectal (30%) Lung (10%) Breast Prostate Ovarian Head and neck Esophageal Gastric Bladder Hepatocellular Renal cell
	Fibrosis Hypertension Osteoporosis Atherosclerosis	Hereditary hemorrhagic telangiectasia	Atherosclerosis			Familial juvenile polyposis

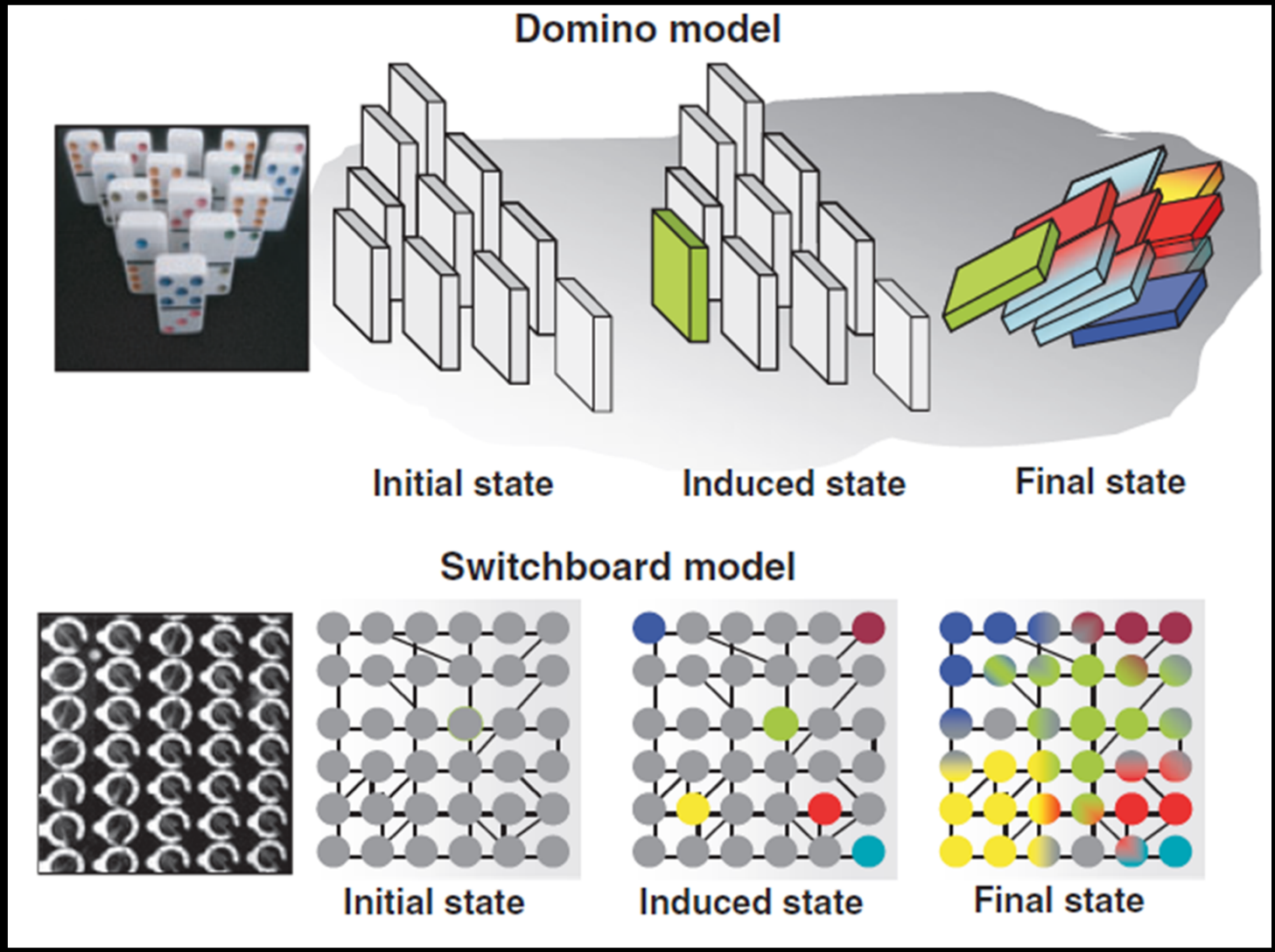
New England Journal Med 2000, 4, 1350





Mullen AC et al Cell 2011, 147, 565 Xi Q et al Cell 2011, 147, 1524

Directing **Differentiation**

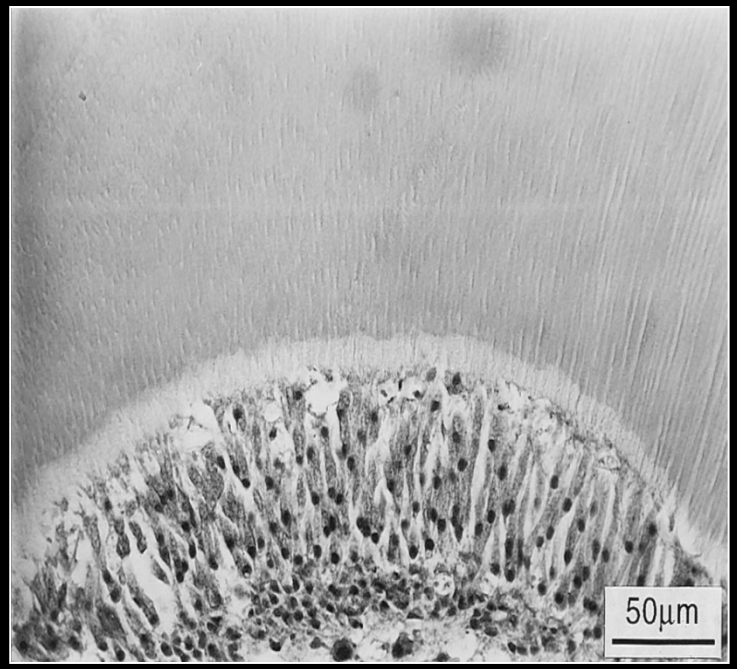


WYSS INSTITUTE

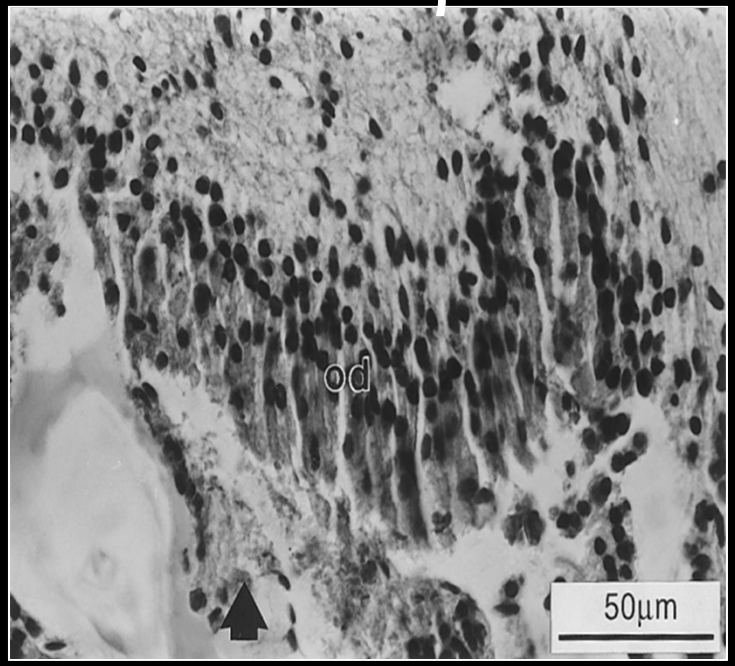
Arany PR & Mooney DJ Oral Dis 2010,17(3):241-51

Applications: LPL activated TGF-β1?

Control



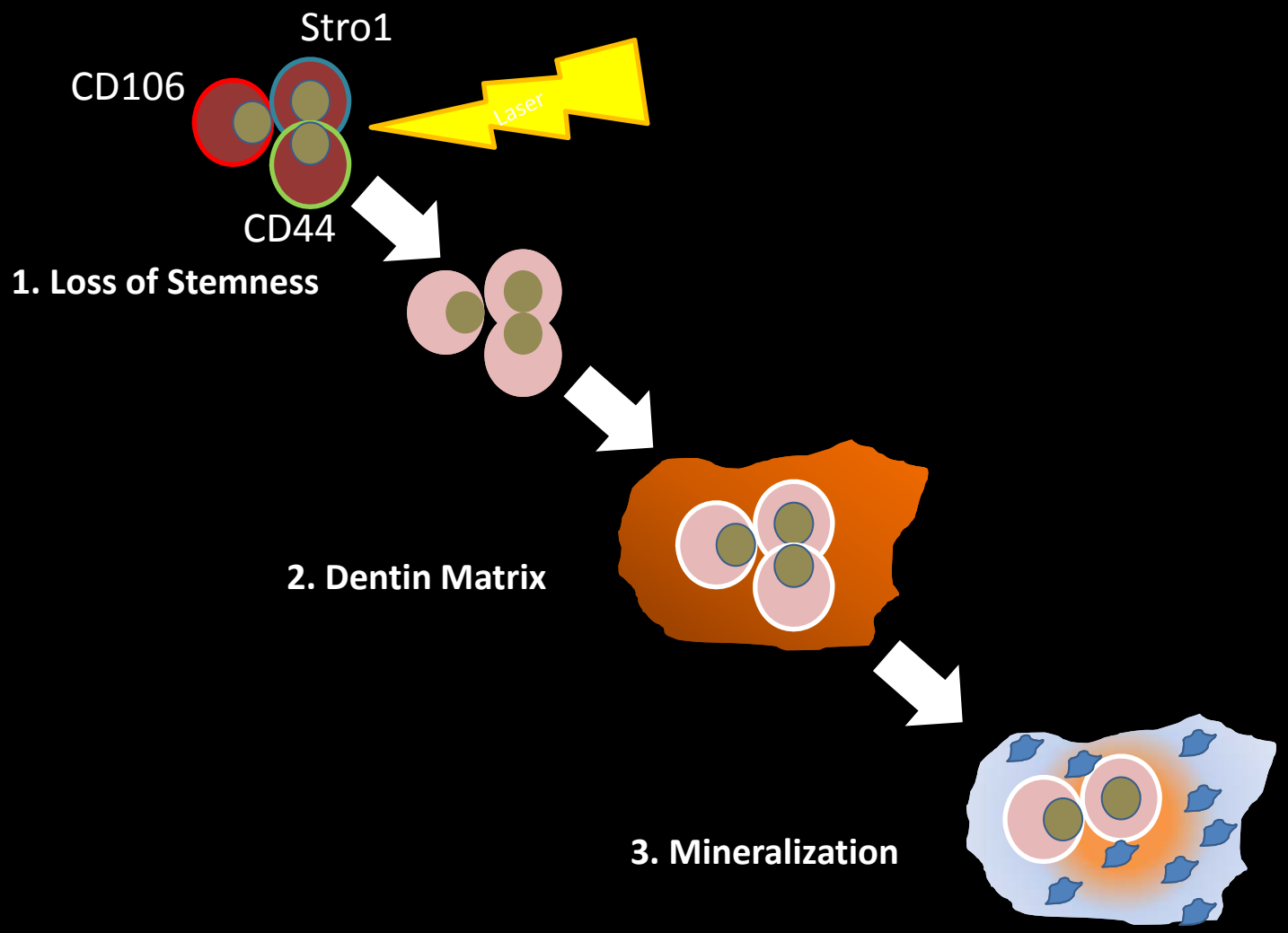
TGF-β



TGF-β1 has a key role in **Dentin biology**

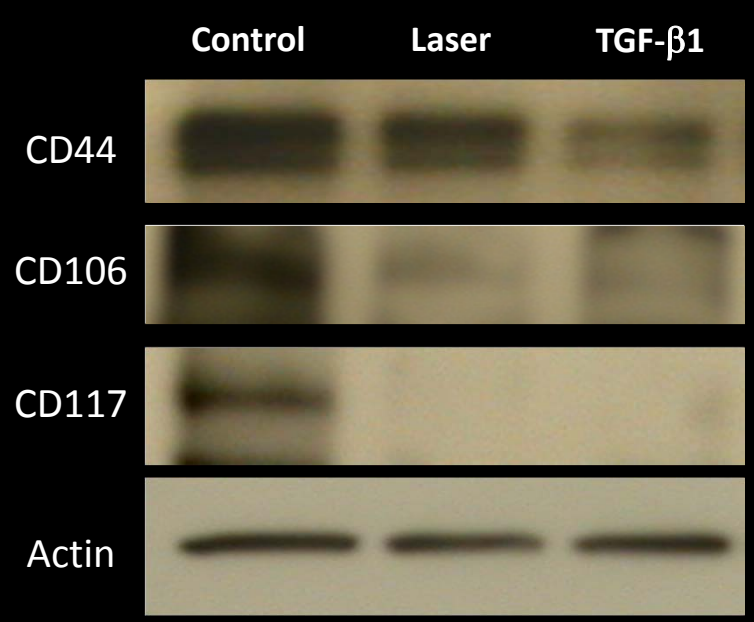
Sloan AJ and Smith AJ **Arch Oral Bio** 1999, 44, 149
D'Souza, RN et al **Eur J Oral Sci** 1998, 106 , 1, 185

Three specific experiments…….

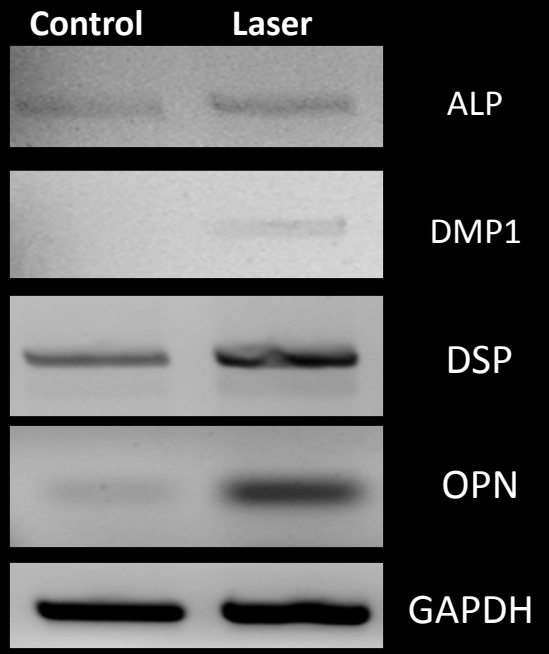


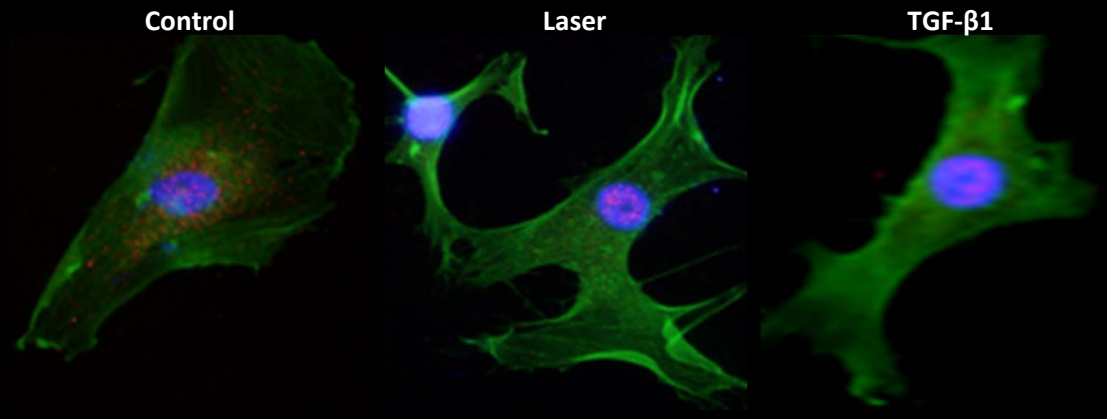
Strategy to *Direct* Dentin Differentiation

Laser: TGF-β1 directs dentin differentiation of hDSCs

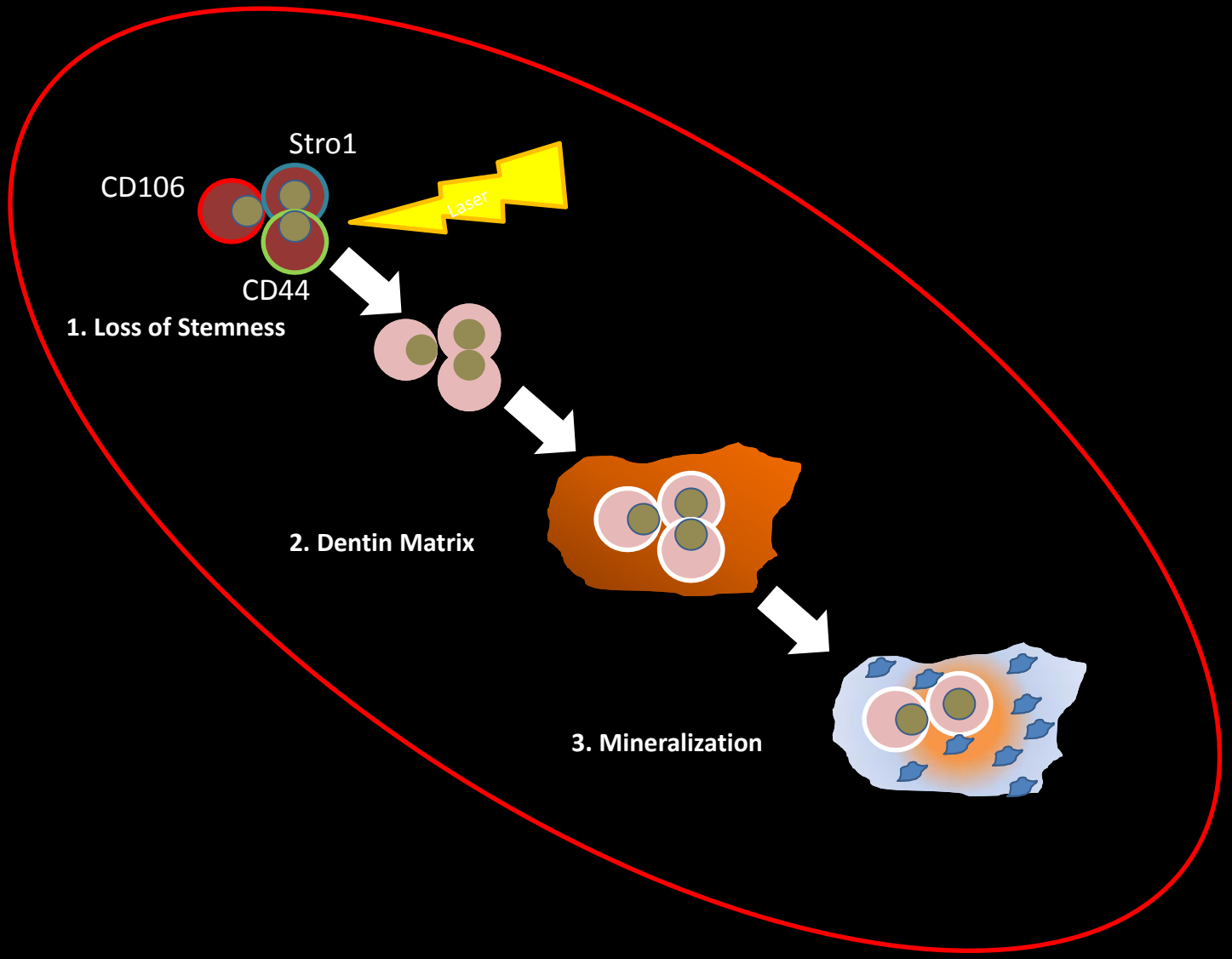


Gronthos S et al PNAS 2000, 97, 13625

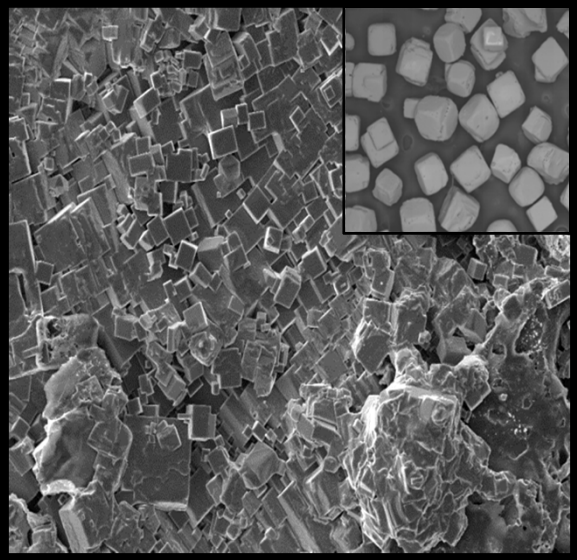


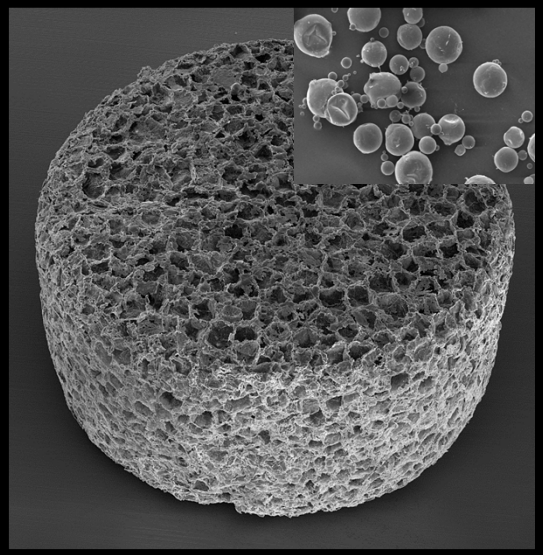


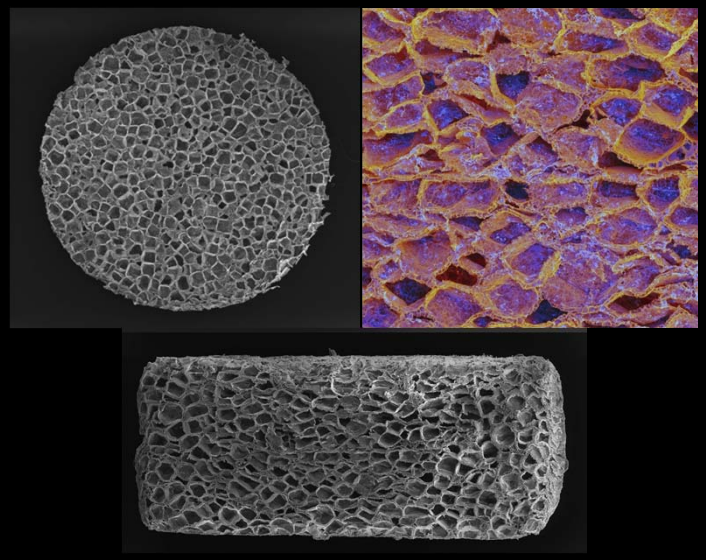
Experimental Strategy

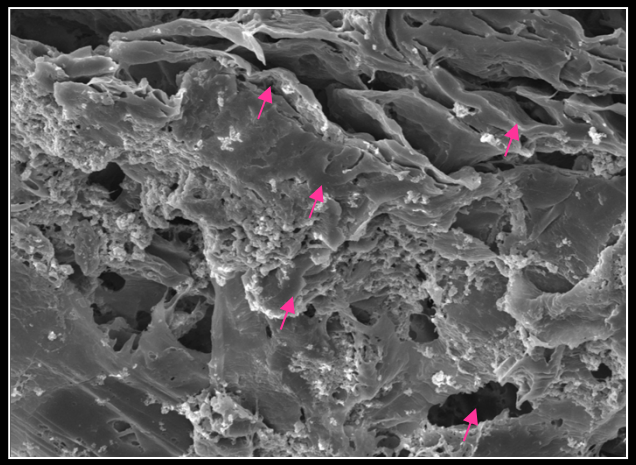


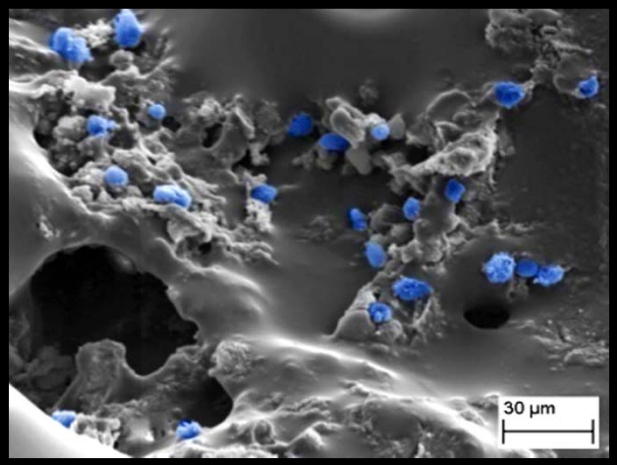
Engineering *in situ* stem cell niches

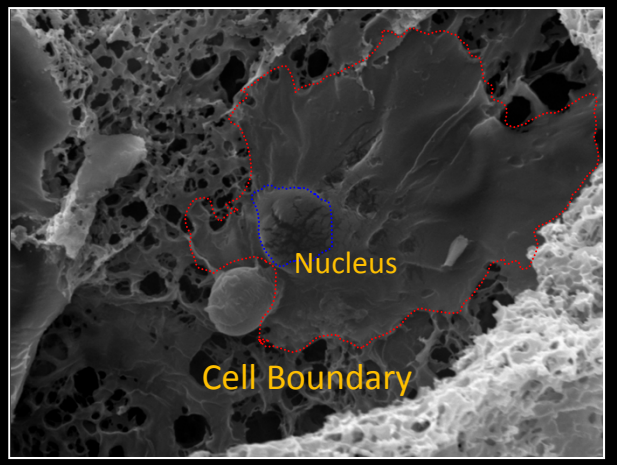






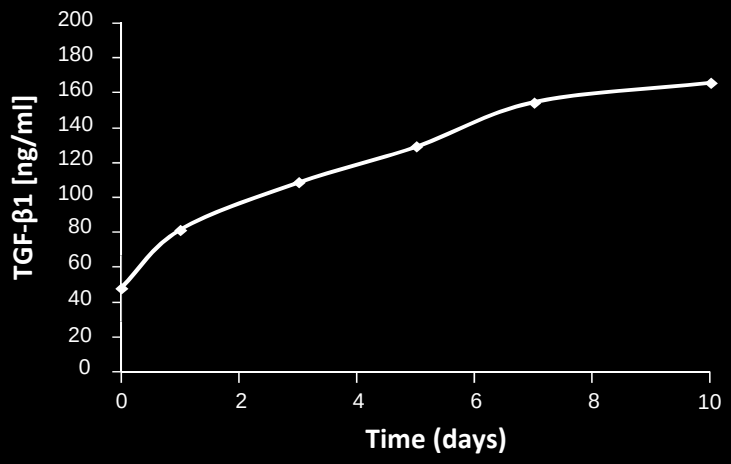


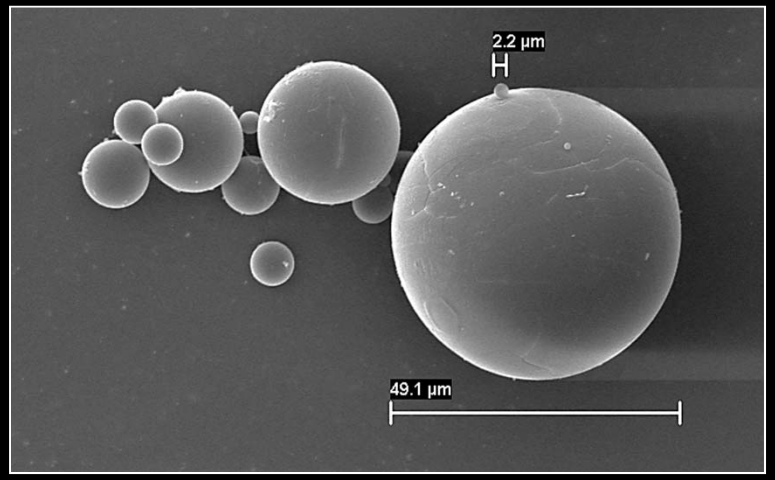


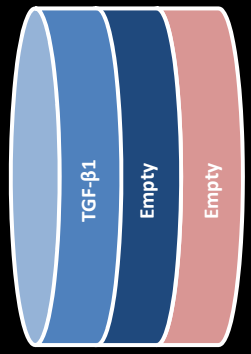


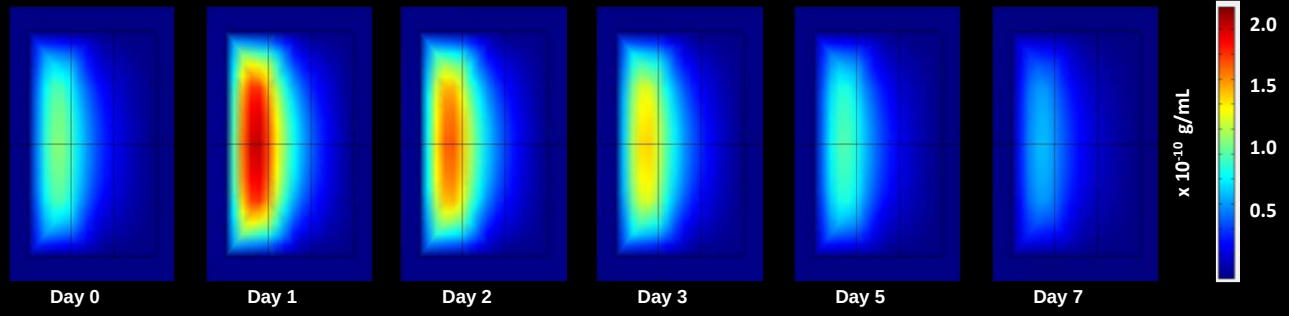
Gas-foaming : salt leaching PLG scaffolds. Mooney DJ *et al* PNAS 1996

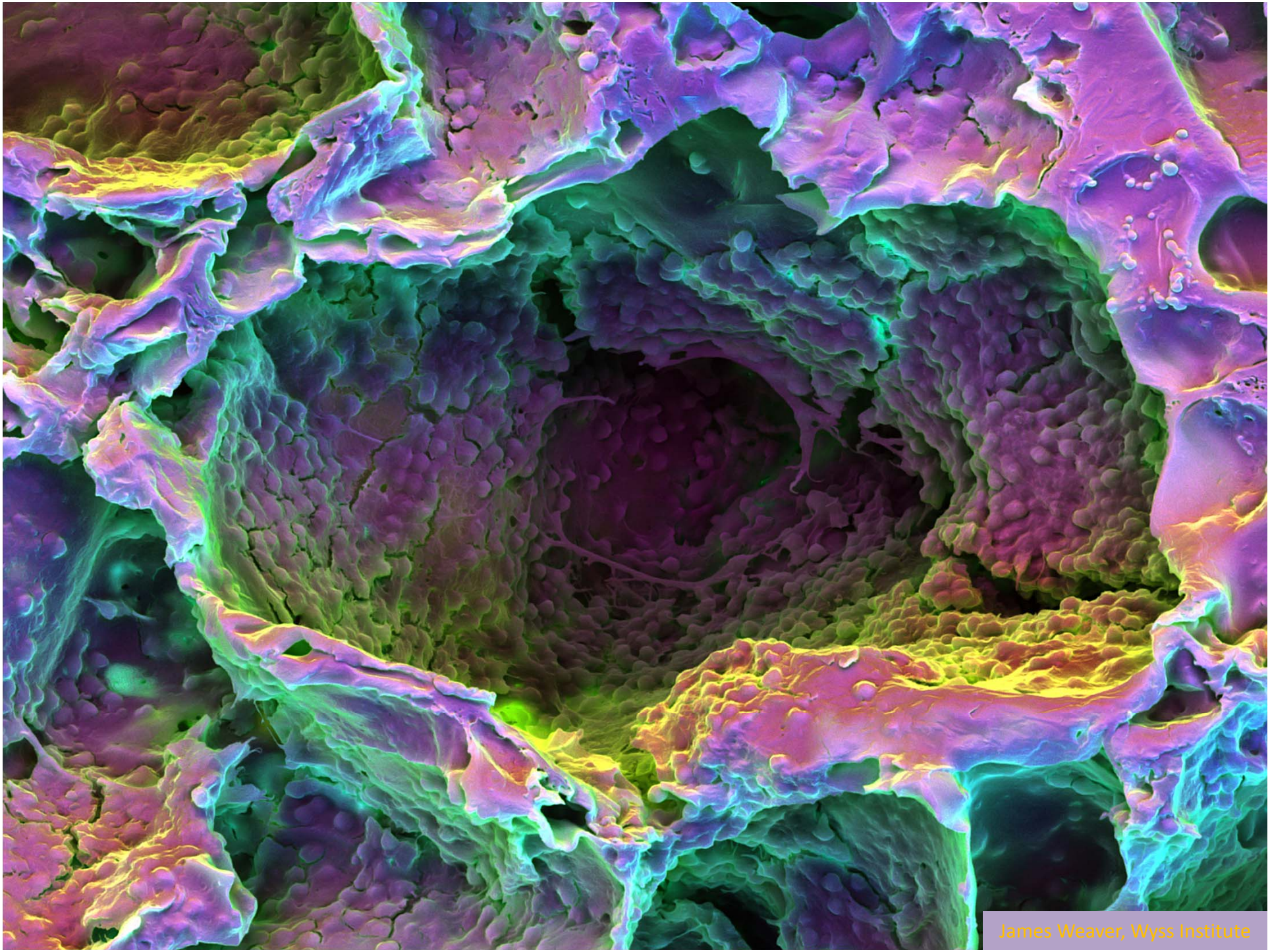
Generating a morphogen niche……










James Weaver, Wyss Institute

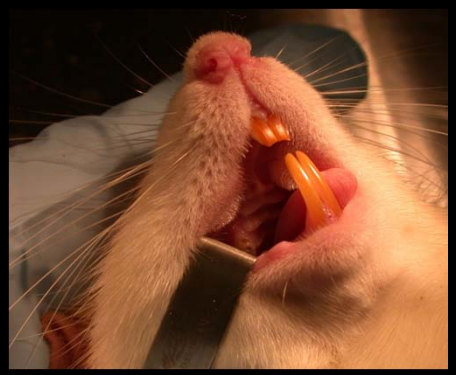
Talk Outline

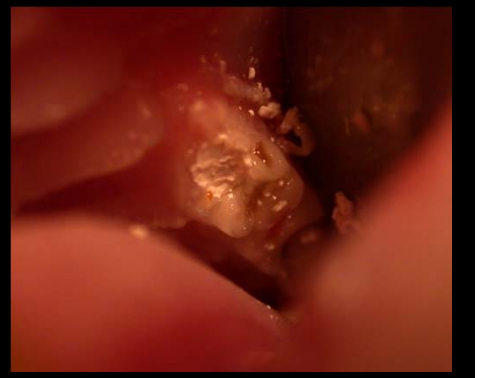
I. Introduction
II. Research (Molecular mechanism)
III. Applications ☞ Lab
☞ Clinic

LPL:TGF-β1 validation *in vivo*

Study Design

- **Control**
- **Laser**
- **DyCal** (Positive control)

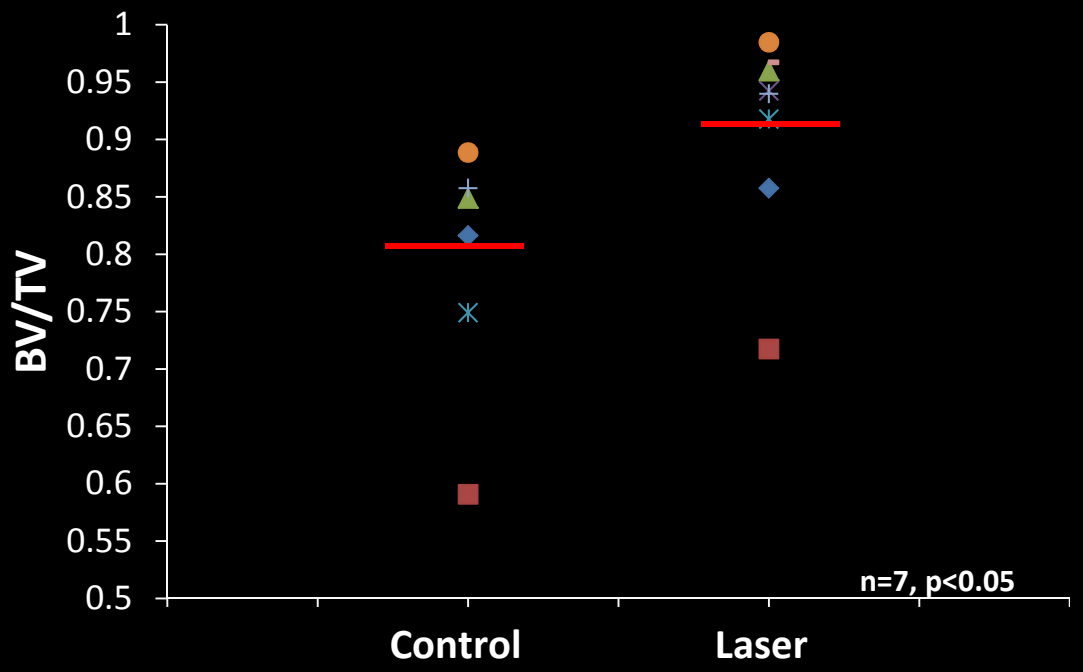


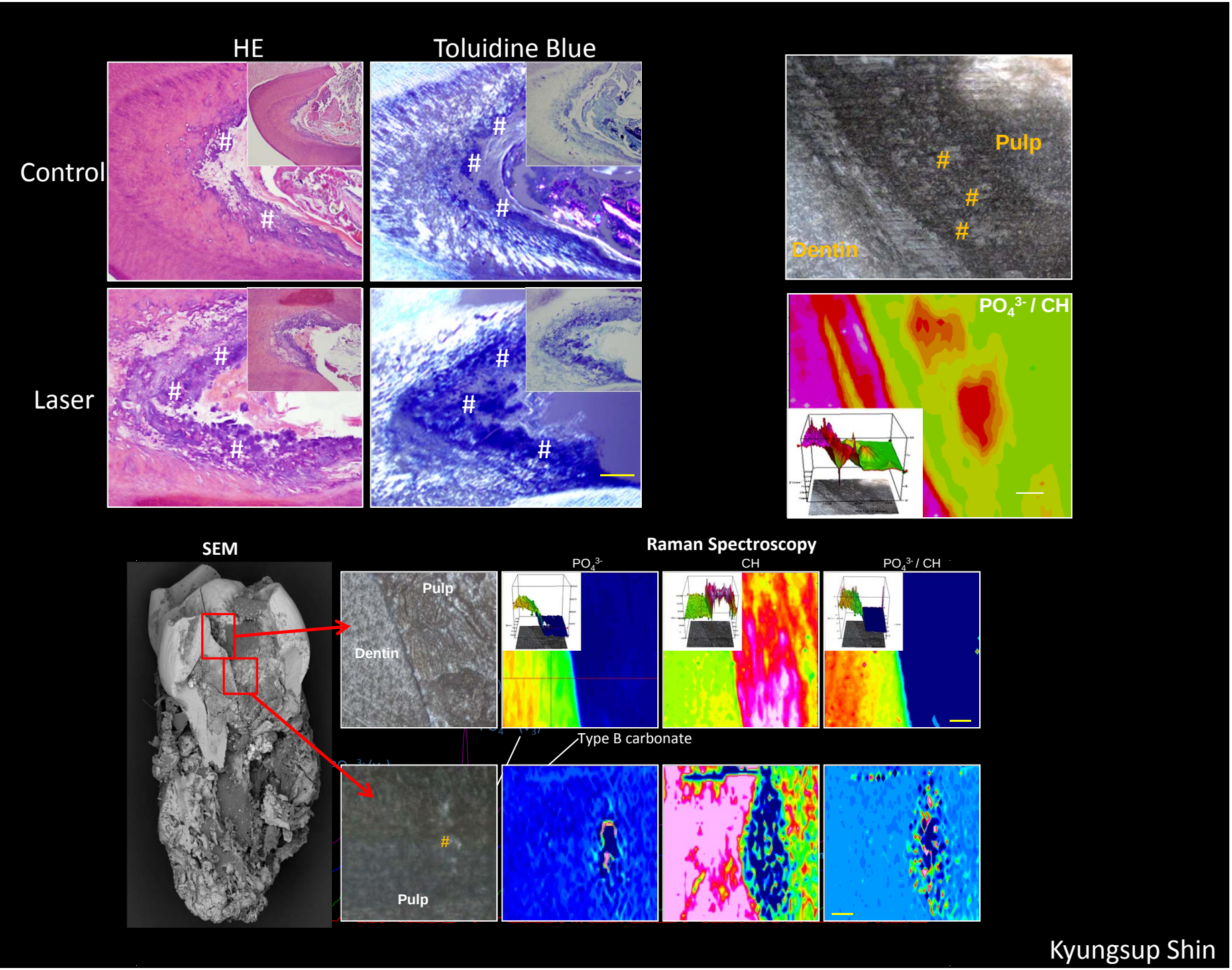
LPL induces Dentin *in vivo*

12 weeks Post-Op

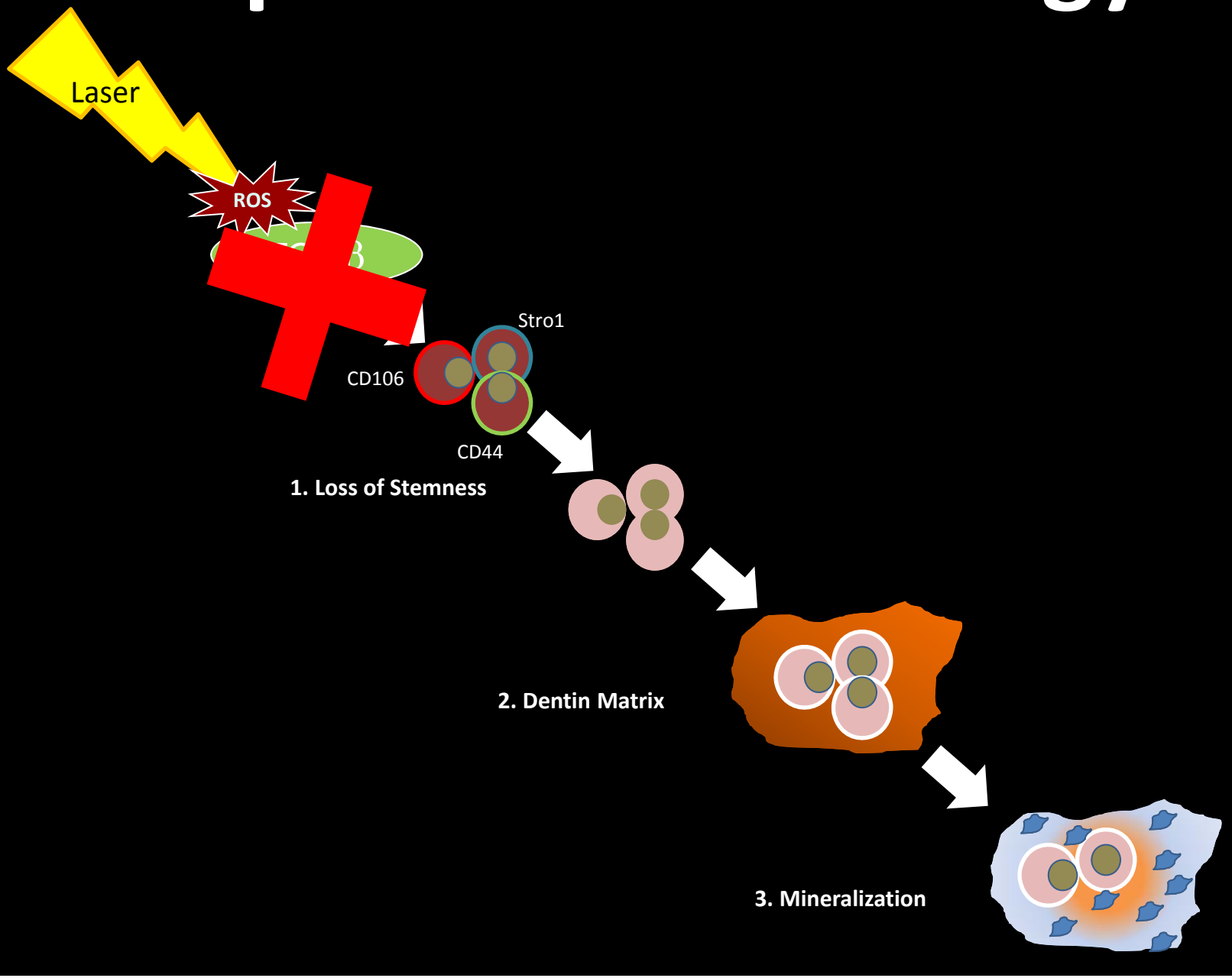
LPL induces Dentin *in vivo*



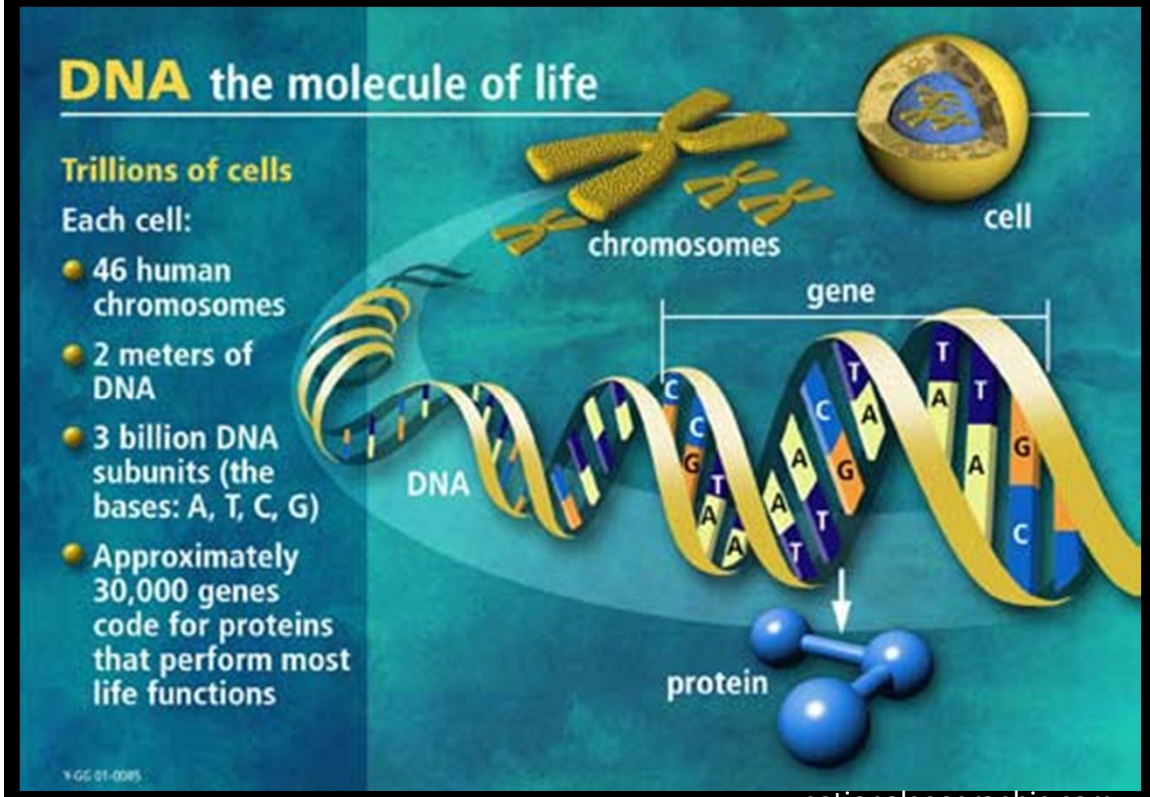
12 weeks Post-Op


HE
Toluidine Blue
Control
Laser
Pulp
Dentin
PO_4^{3-} / CH
SEM
Raman Spectroscopy
PO_4^{3-}
CH
PO_4^{3-} / CH
Pulp
Dentin
Type B carbonate
Pulp
Kyungsup Shin

Experimental Strategy



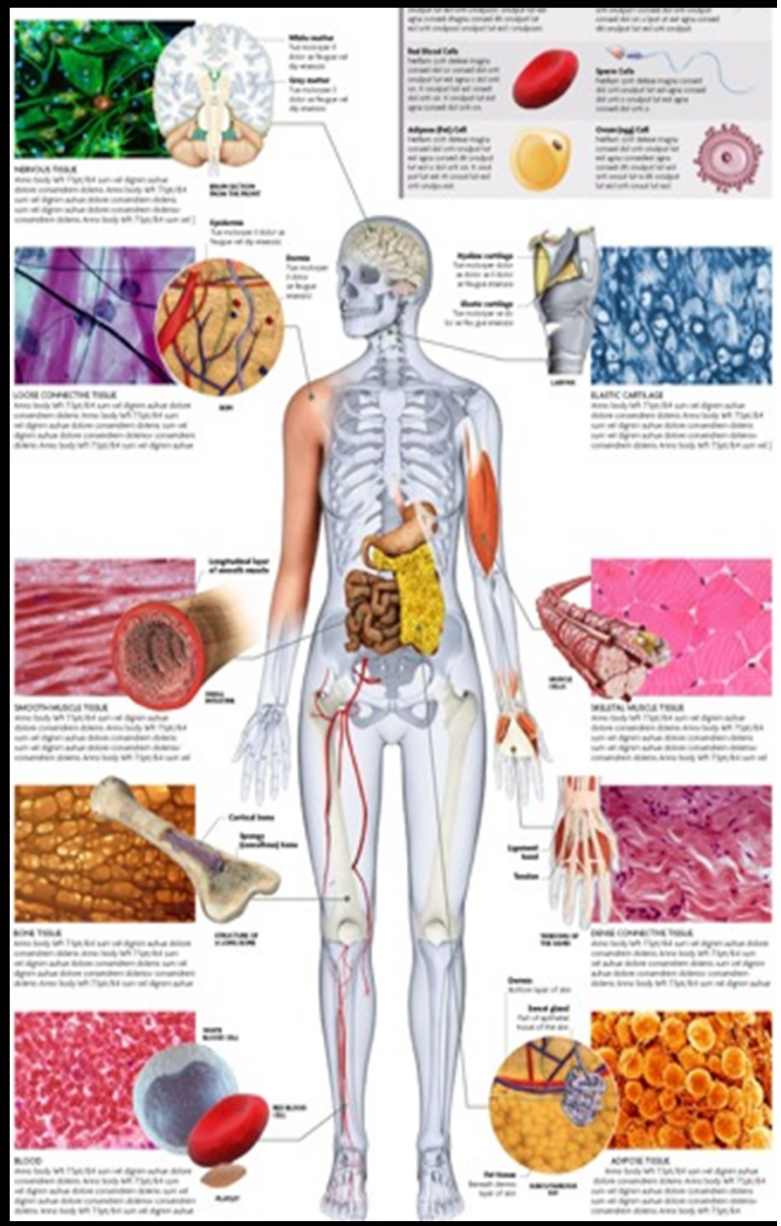
Genetic Engineering Strategy



nationalgeographic.com

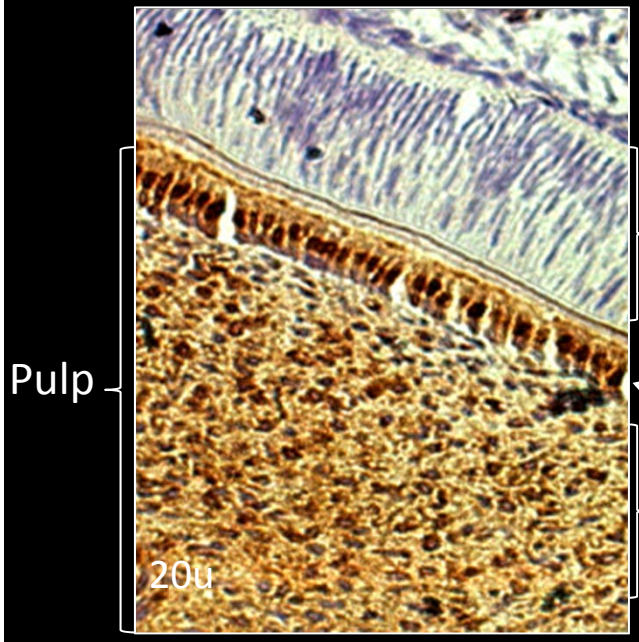
Every tissue is defined by a genetic profile

- Muscle → Muscle Specific Actin
- Melanocyte → Melanin
- Enamel → Amelogenin
- Dentin → Dentin Siaolophosphoprotein **(DSPP)**

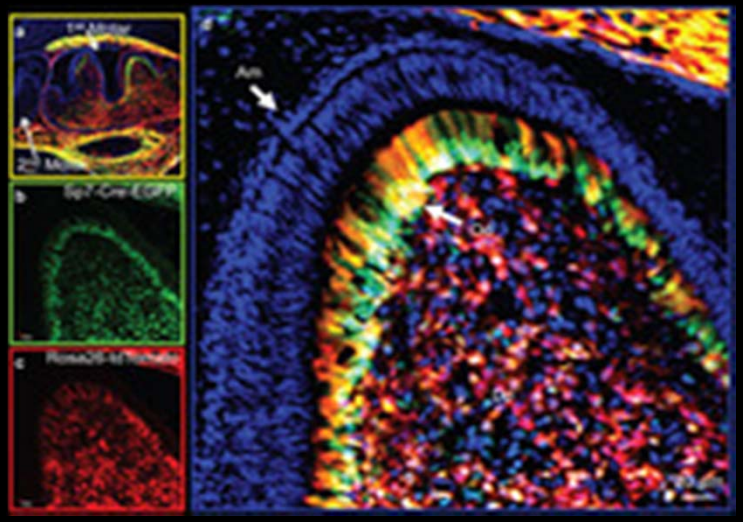


http://www.cashou.com/portfolio/human_body/

Genetic Engineering Strategy

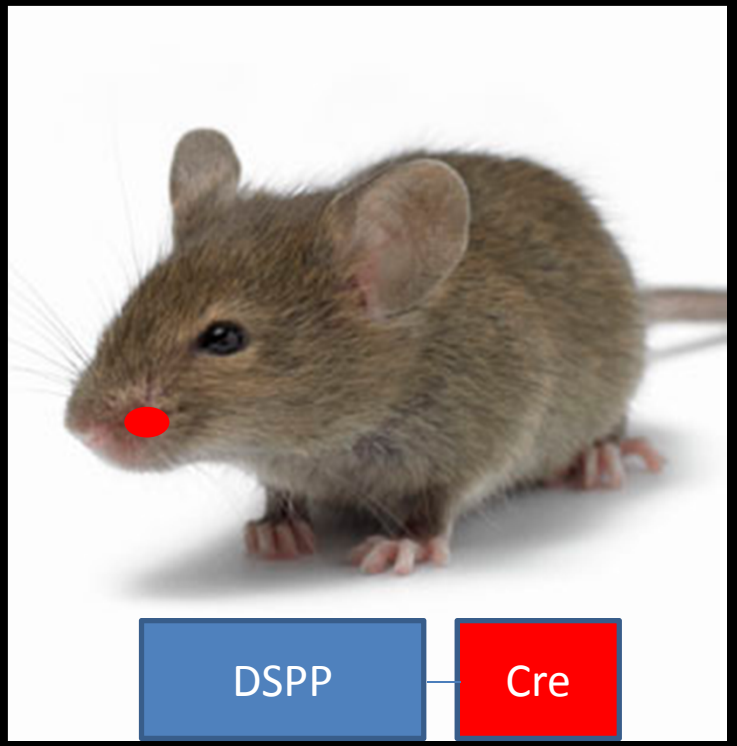


DSPP expression

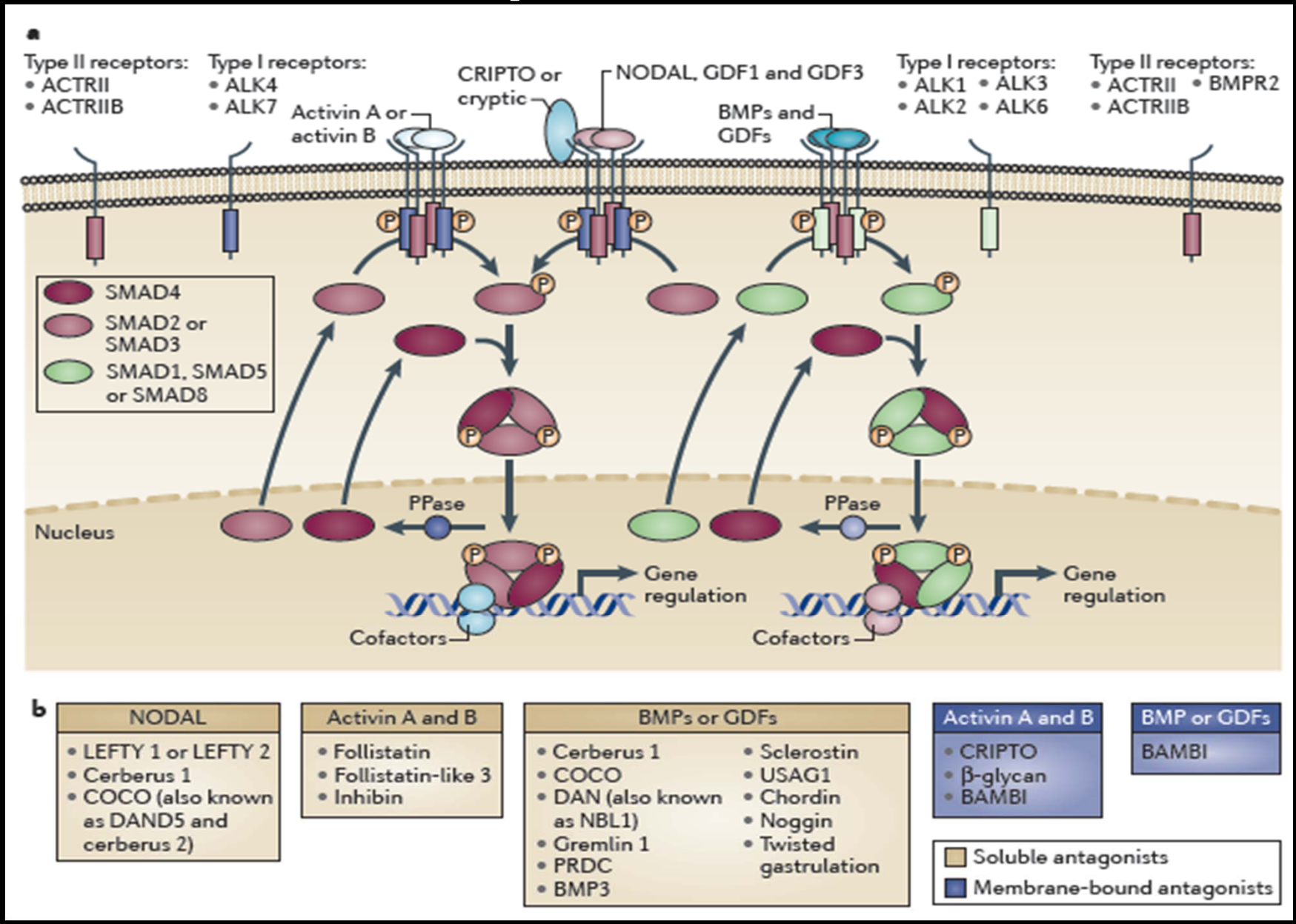


Intl J Oral Sci 2013, 5, 75–84

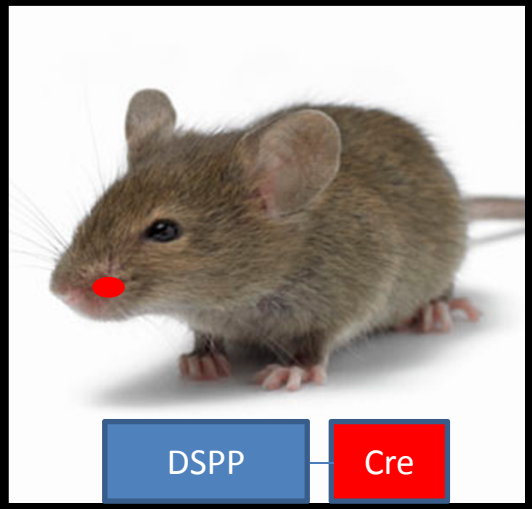
Odontoblasts & Dental Stem Cells



TGF-β Signaling



CoKo mice: All dentin producing cells are TGF-β insensitive



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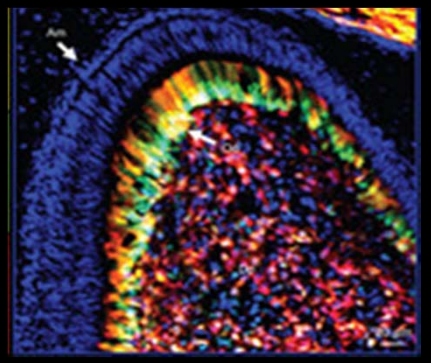
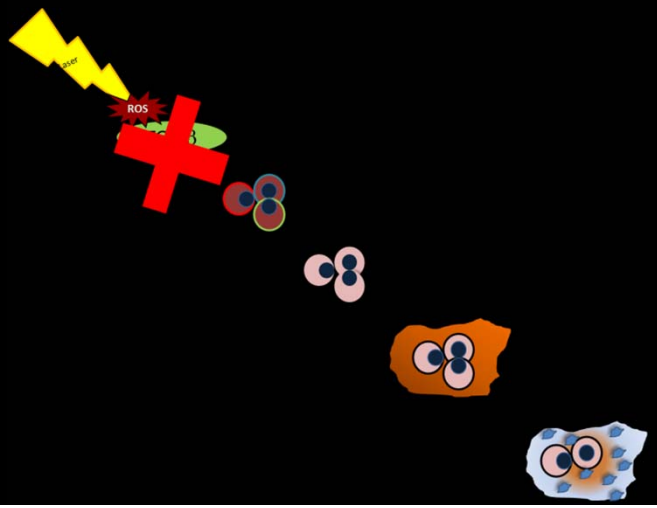
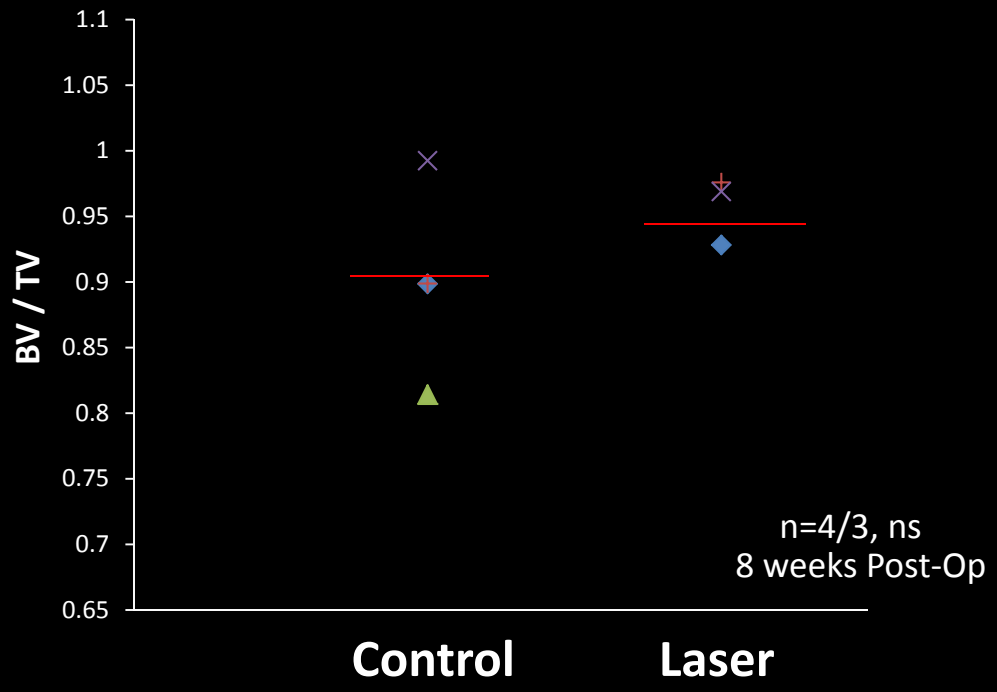
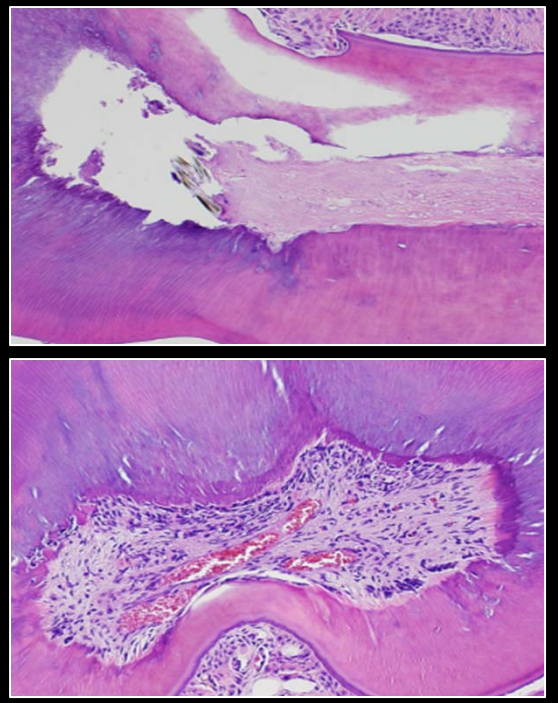




Laser induces Dentin repair via TGF-β





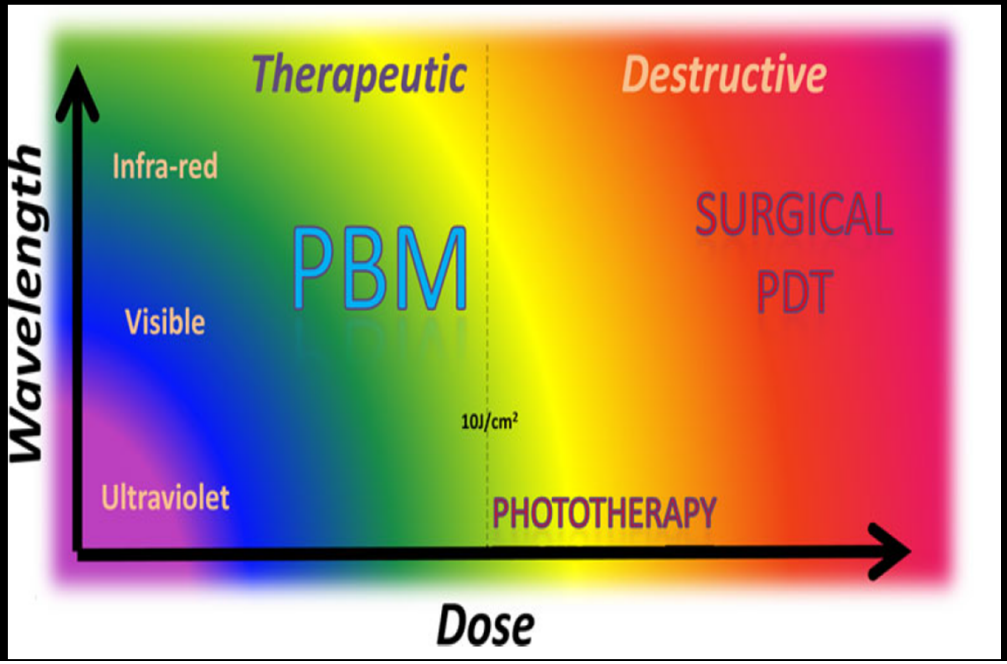
Control (coKO)

Laser (coKO)

8 weeks Post-Op

Conclusions….

1. Low power lasers generate Reactive Oxygen Species
2. LPL activates <u>endogenous</u> latent TGF-β1 (*A mechanism*)
3. LPL directs resident dental stem cell differentiation (TGF-β1)



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