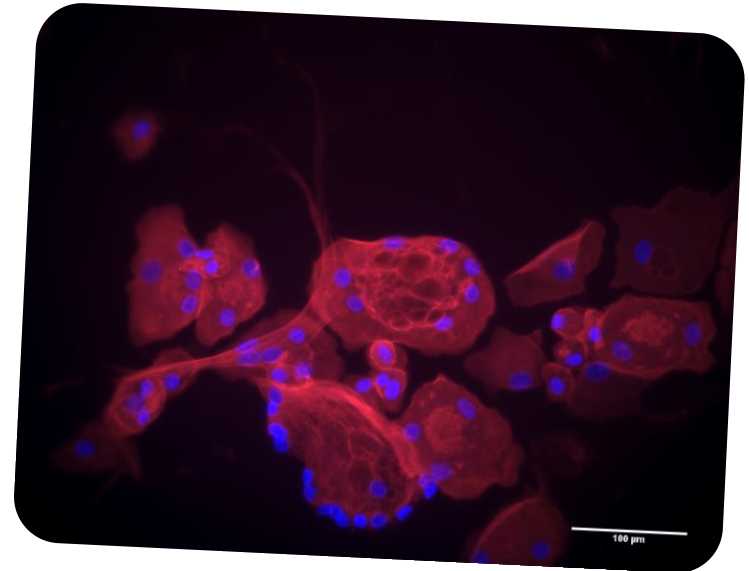# *Who manipulates who in dysregulated mineralised tissue resorption?*
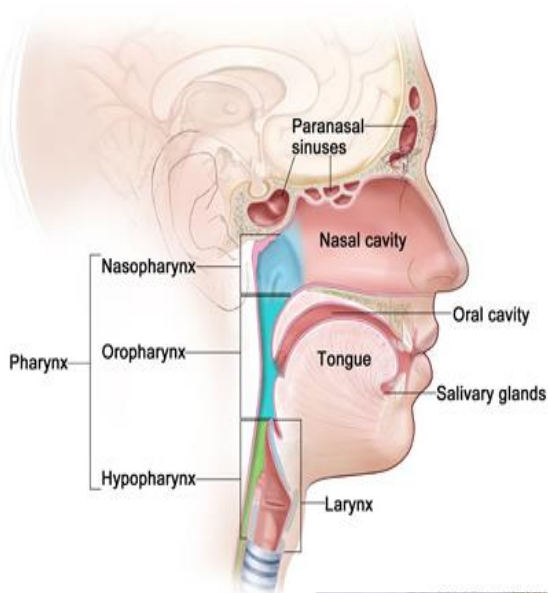
Dr Gurå Therese Bergkvist MRCVS

Senior Lecturer in Veterinary Anatomy &

Clinical Research Associate of The Roslin Institute

Clinical diseases involving mineralised tissues
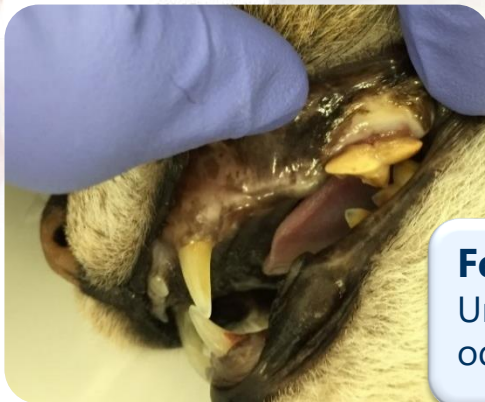

Head and Neck Cancer Regions

Shared risk factors
Shared environments
Shared poor prognosis
Shared molecular markers

Feline oral squamous cell carcinomas
Locally aggressive, commonly invade into bone.
Highjacks the osteoclasts to invade?

Feline tooth resorption
Unknown cause, reactivation of tooth resorption by
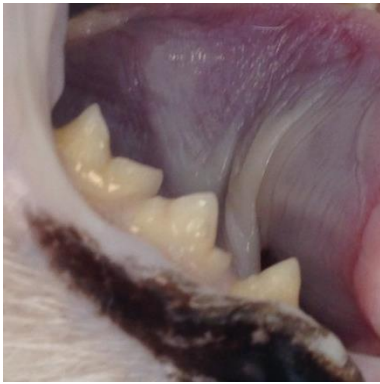odontoclasts years after shedding of milk teeth

A. Feline tooth resorption –
A study of odontoclast dysregulation in feline tooth resorption

Feline tooth resorption

Healthy teeth

Early stage of TR in molar

Multiple teeth affected, extensive tissue loss

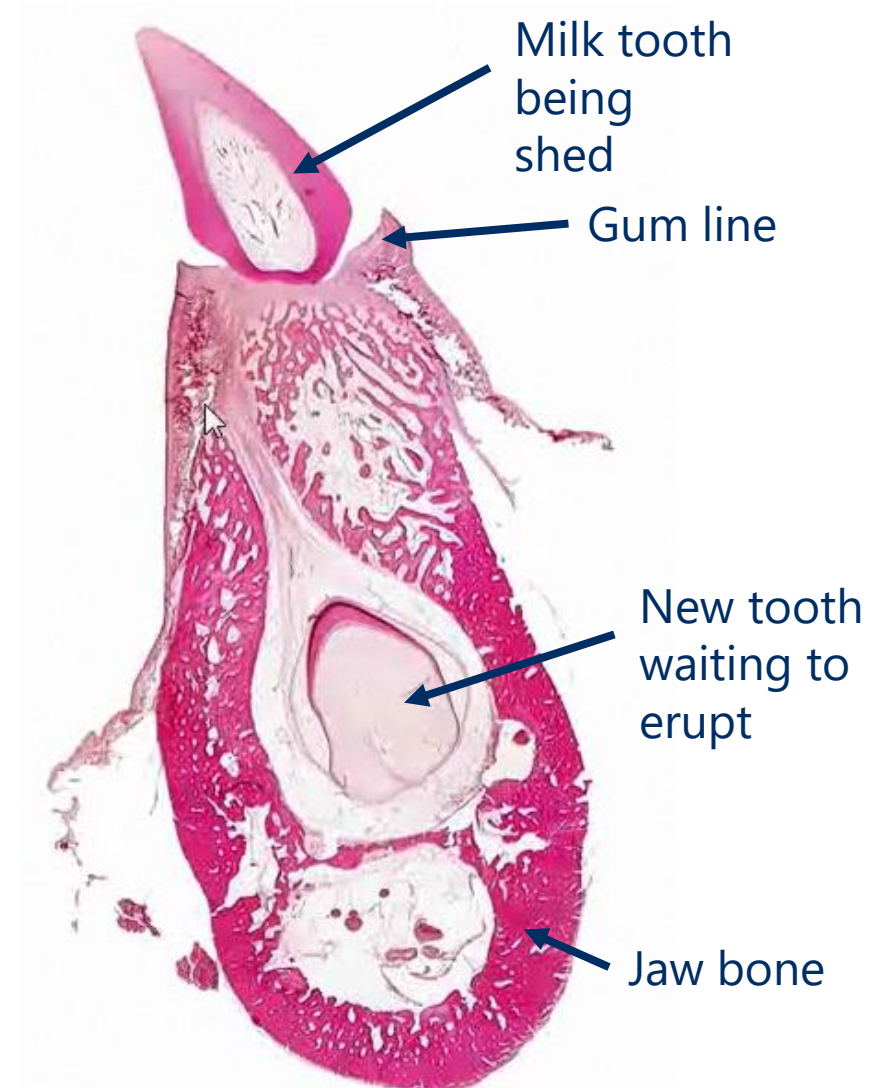**Final stage of TR** Loss of dentition

Tooth shedding

Replacement of milk teeth by permanent dentition

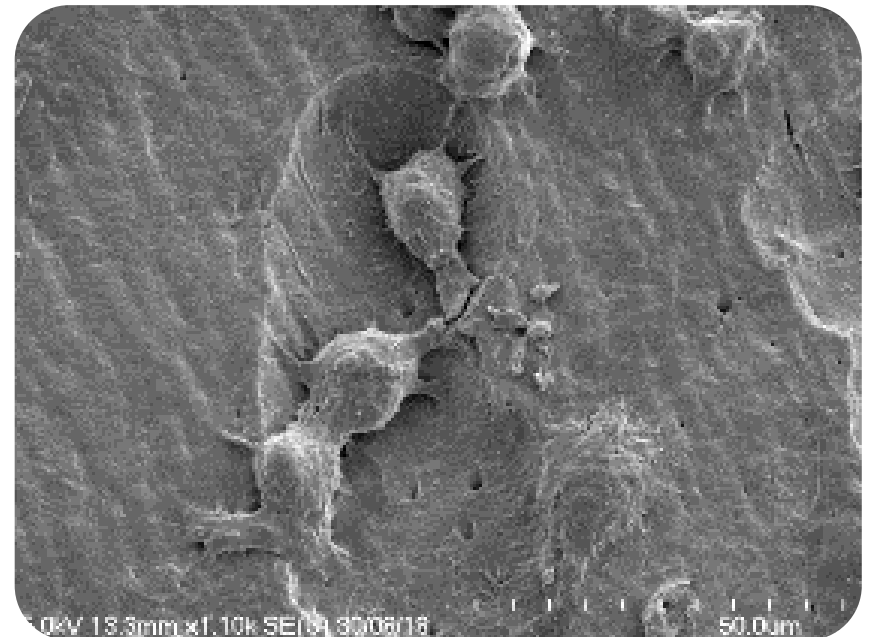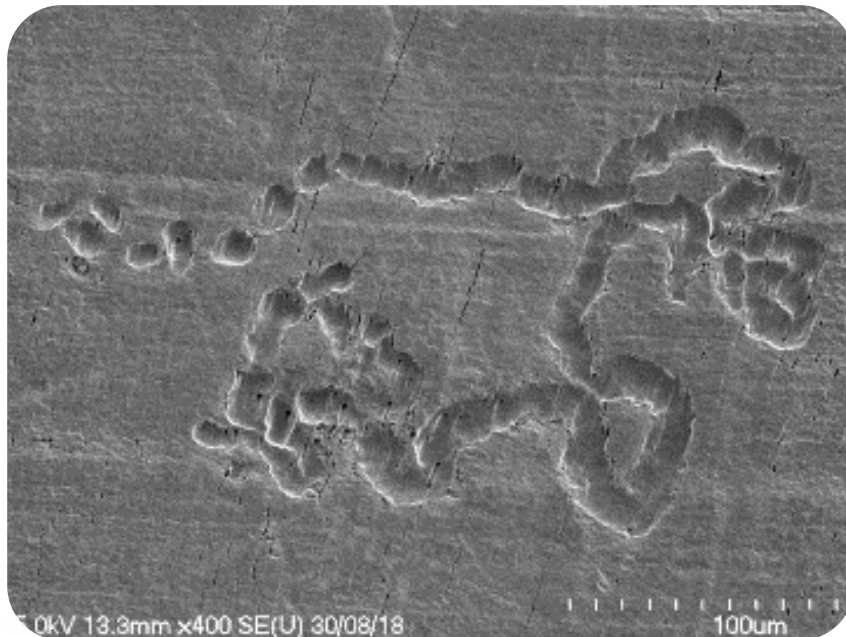Tooth root resorption of milk teeth

Underlying adult tooth not resorbed

How is this possible?

Milk tooth being shed

Gum line

New tooth waiting to erupt

Jaw bone

The cells that can resorb mineralised tissues like dentine, enamel, and bone:

Odontoclast (in teeth) or Osteoclast (in bone)

How does the odontoclast know to resorb the milk teeth and not the adult teeth right next to it?

Periodontal Ligament (PDL) cells & the cells of the microenvironment
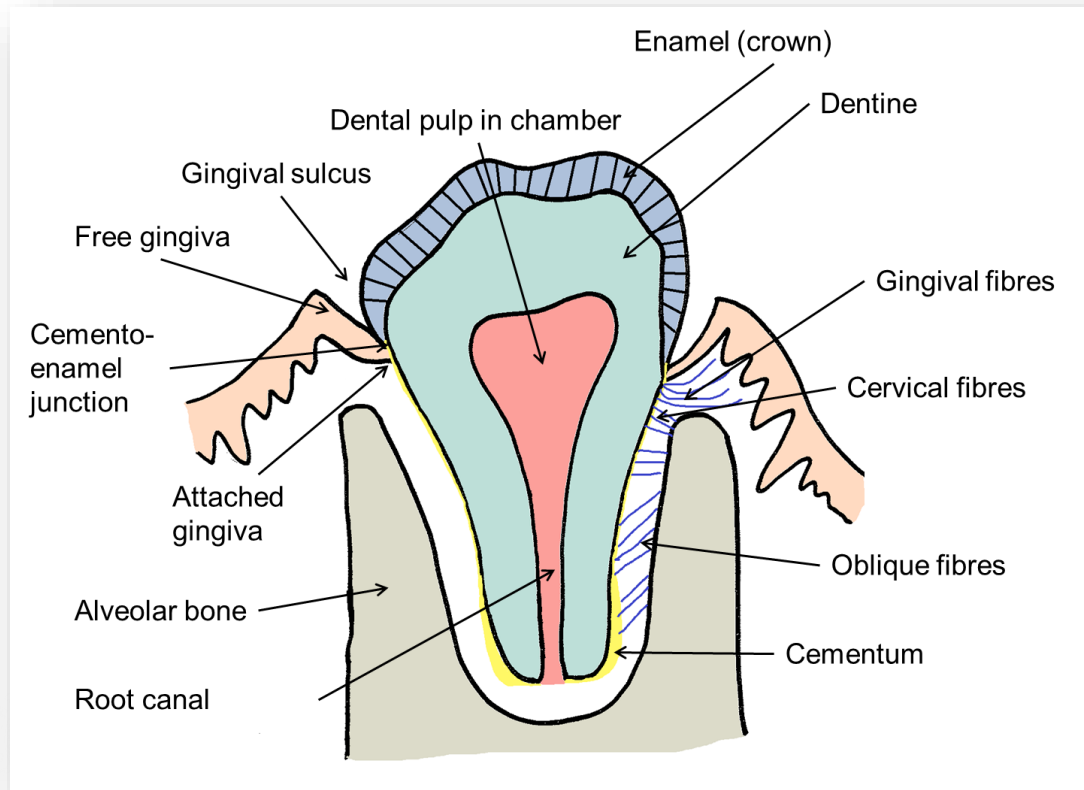
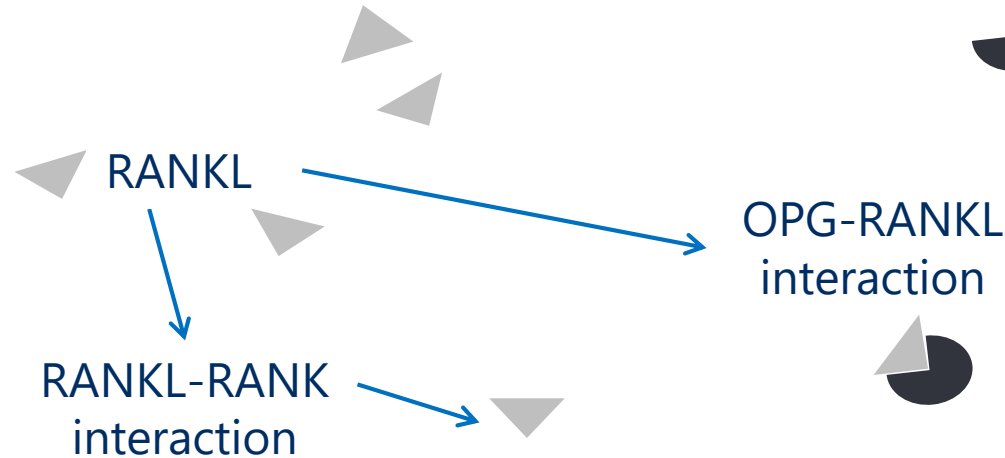**The PDL cells use a system of communication called the RANKL/RANK/OPG system**

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RANKL/RANK/OPG SYSTEM

RANKL expressed from PDL of milk teeth >>permanent teeth

RANKL

OPG-RANKL interaction

OPG is a decoy receptor so this interaction causes no activation. Produced by permanent teeth

RANKL-RANK interaction

RANK

⊕ Stimulation of tooth resorption
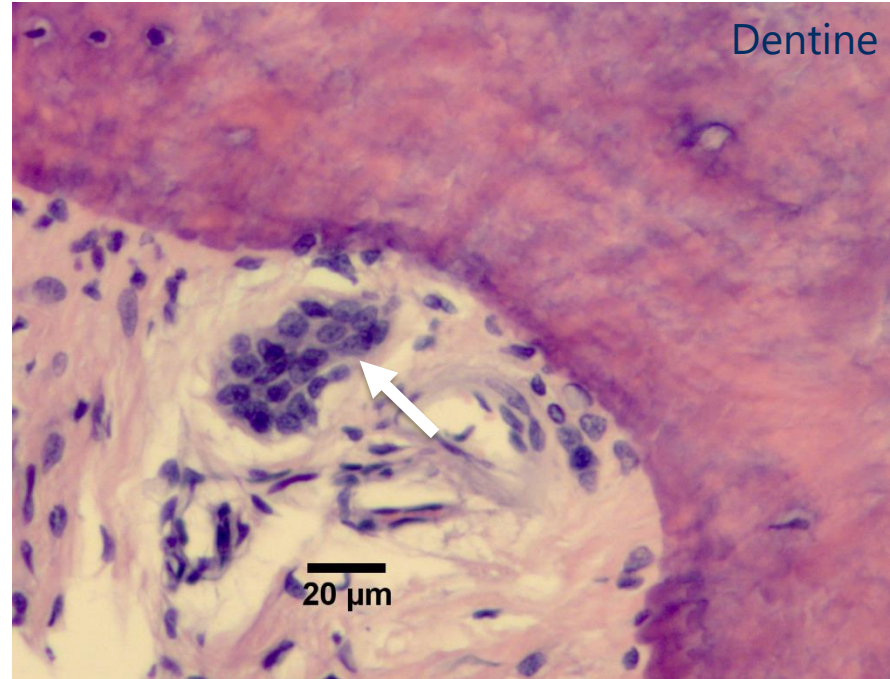
Odontoclast

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It doesn't always work……feline tooth resorption

Odontoclast in molar with advanced stage TR

Dentine

20 μm

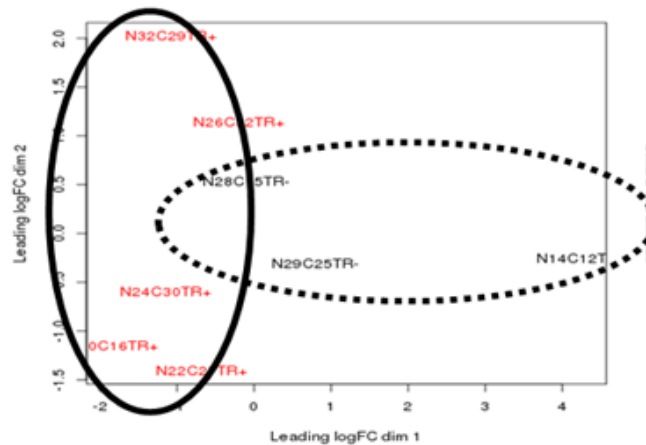# ……sometimes odontoclasts become reactivated later in life

RNA seq study
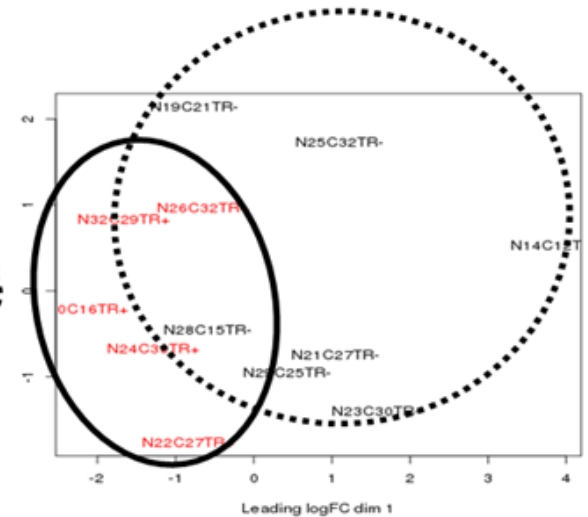
A = teeth from TR affected vs. TR free cats

B = TR affected teeth vs. TR free teeth

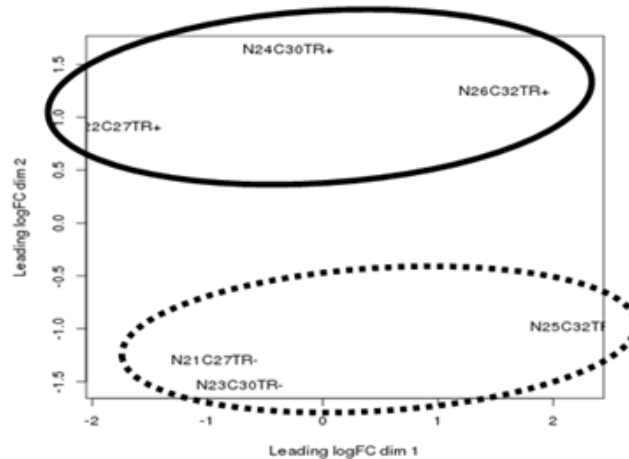C = TR affected teeth and TR free teeth from same cat

A. TR -/+ cats

B. TR -/+ teeth

C. Paired TR -/+ teeth

C showed 16,307 differentially expressed genes

RNA seq study results – Analysis

Abbreviation	Name	Function
MMP9	Matrix Metallopeptidase-9	Role in ECM degradation
P2XR4	P2X purinergic receptor 4	Ligand-gated cation channel
SPI.1	Spi-1 proto-oncogene	Transcriptional activator, possible role in differentiation/activation of osteoclasts and odontoclasts
CTSK	Cathepsin-K	Expressed by osteoclasts, role in ECM degradation
RANKL	Receptor Activator of Nuclear Factor κ-B Ligand	Triggers osteoclast and odontoclast differentiation/activation
OPG	Osteoprotegerin	Decoy receptor for RANKL

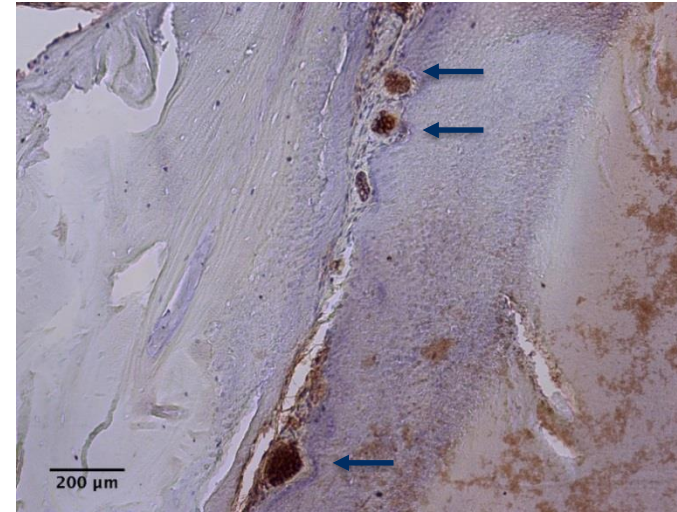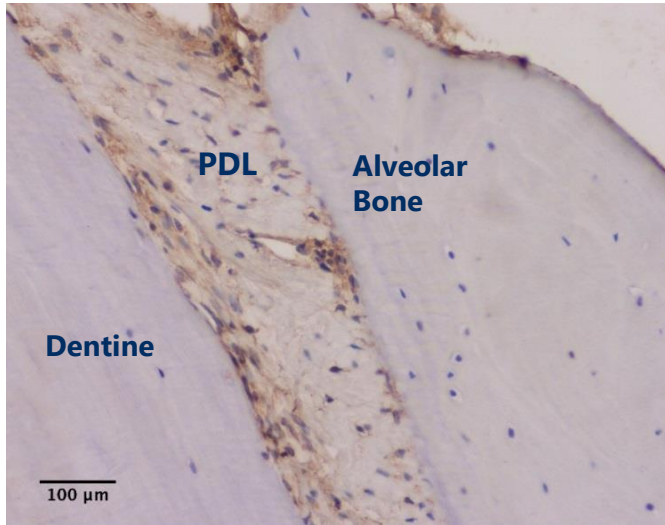# MMP9 and P2XR4 were significantly upregulated
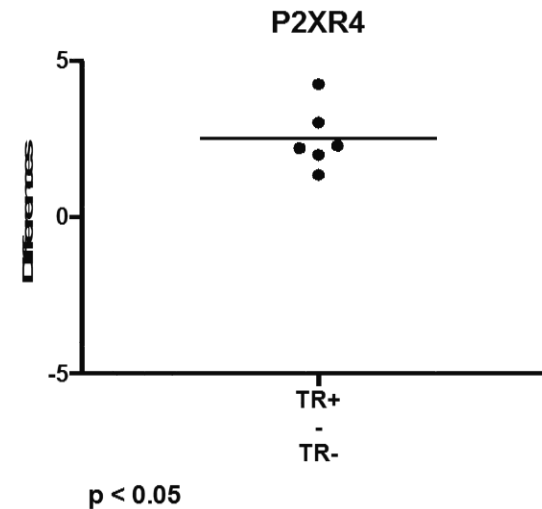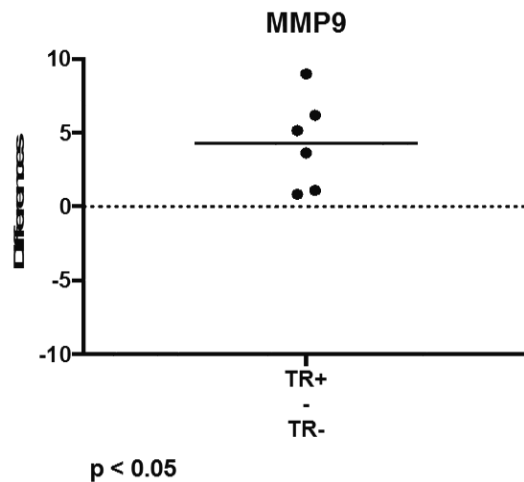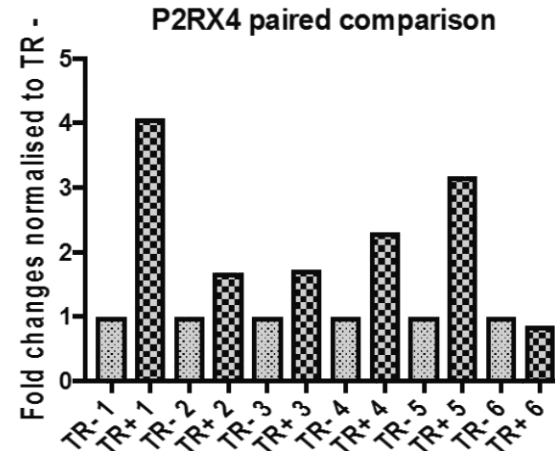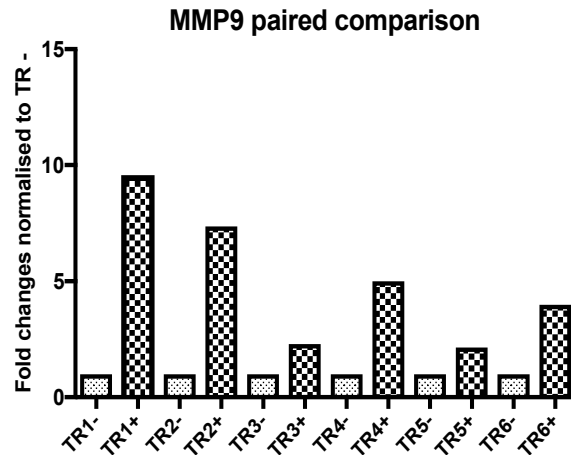
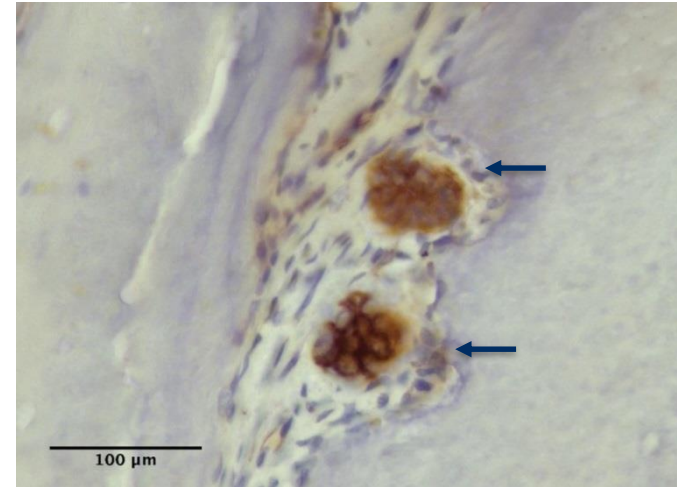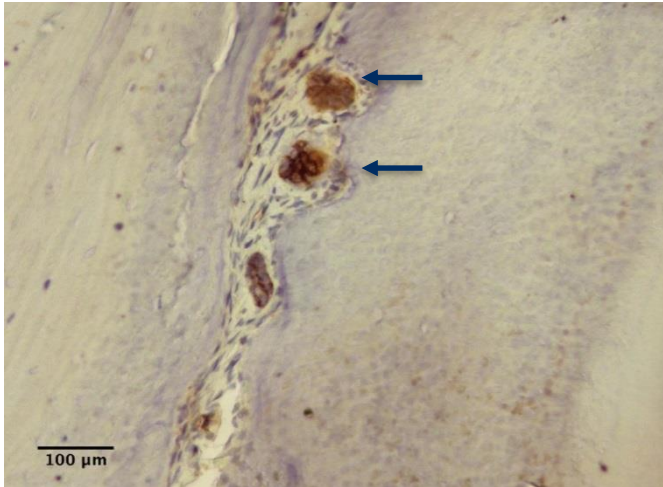# H&E sections of healthy tooth & tooth resorption lesion
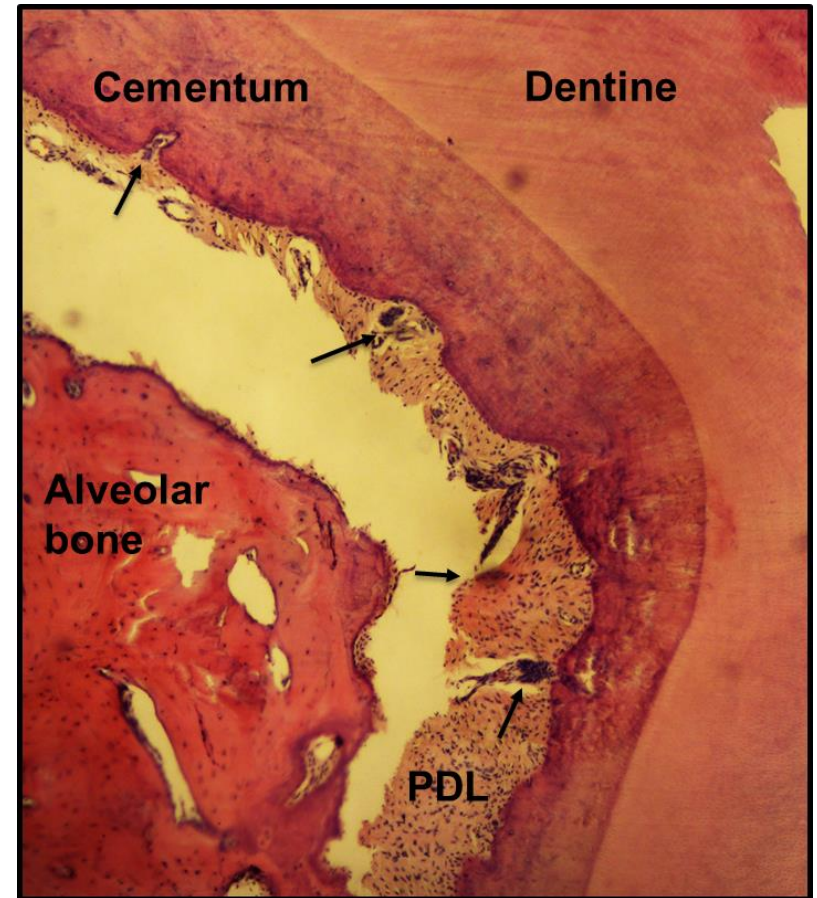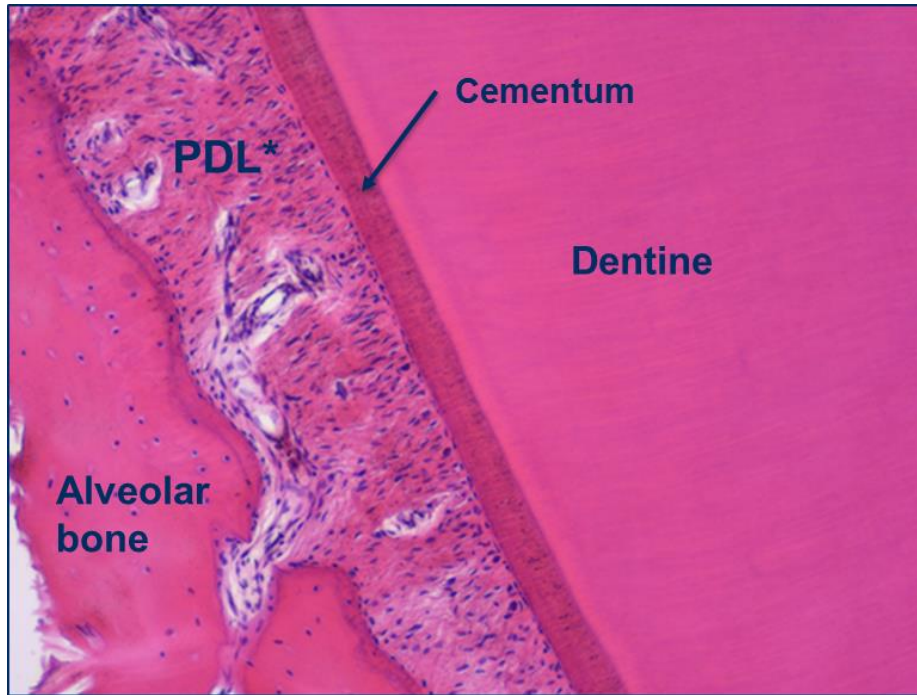
MMP9 positive odontoclasts in resorption pits
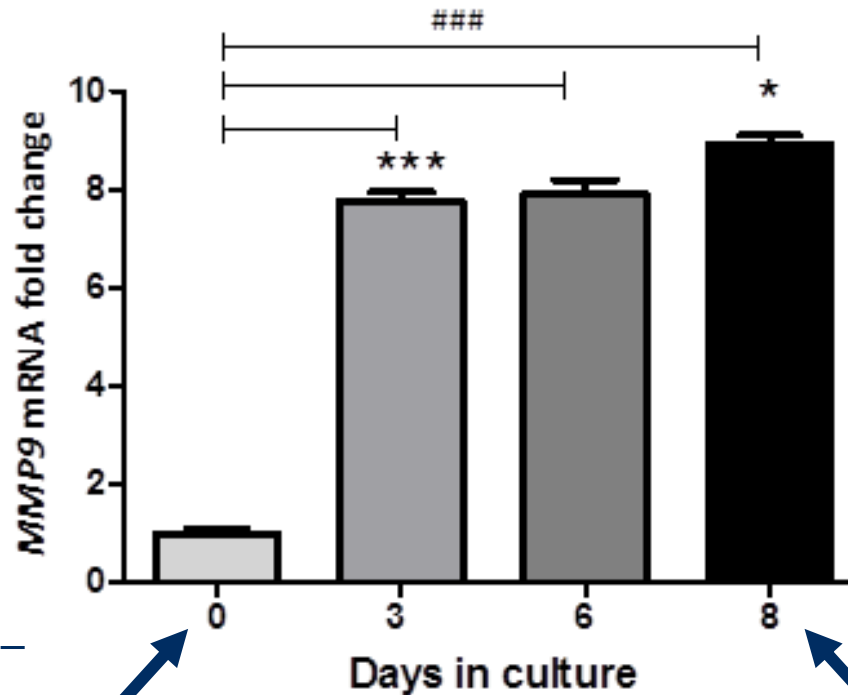
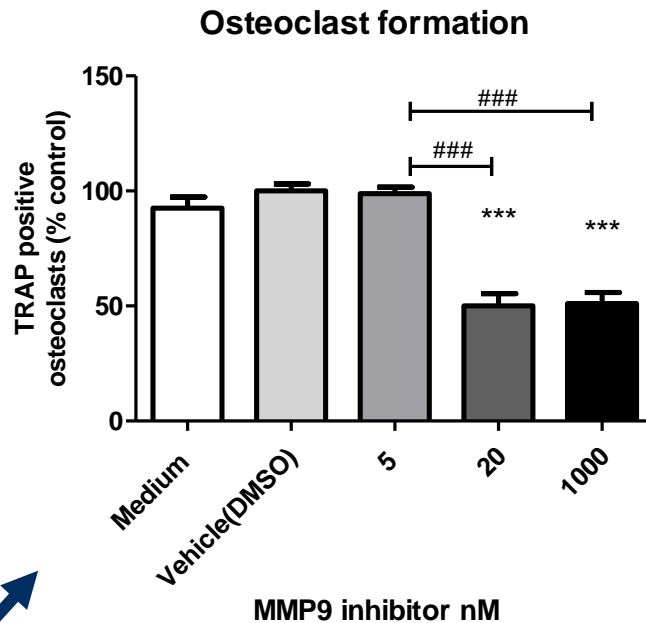# MMP9 expression increases rapidly at an early stage during osteoclast differentiation

Bone marrow cells – enriched for osteoclast precursors (myeloid origin)

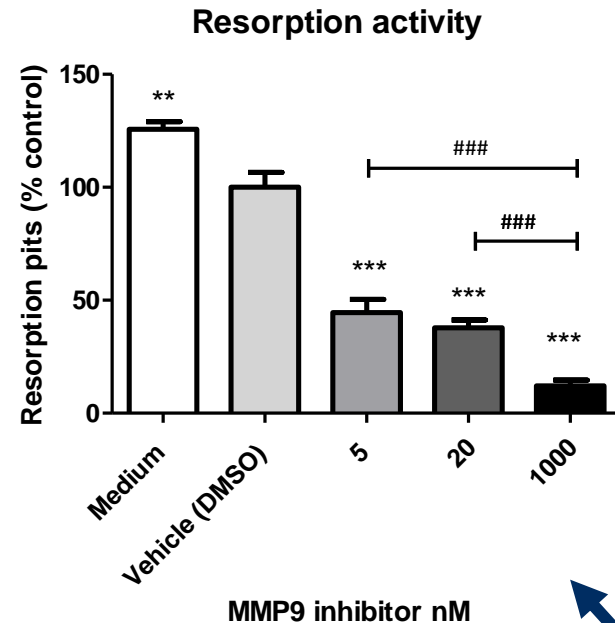Terminally differentiated osteoclasts

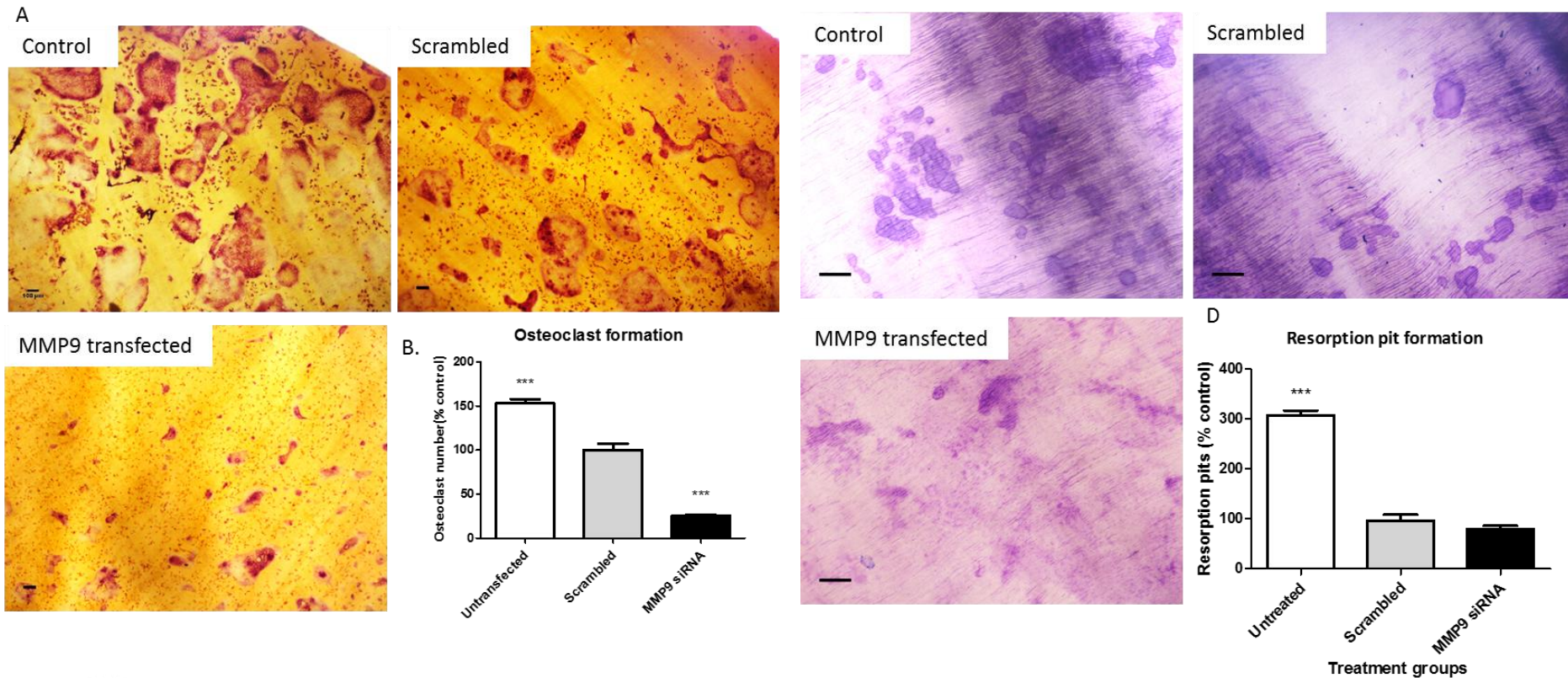# MMP9 inhibitor reduced osteoclast formation and resorption activity

Number of osteoclasts formed was reduced

Osteoclast resorption activity was reduced

MMP9 siRNA reduced osteoclast formation but not resorption activity

Number of osteoclasts formed was reduced

Osteoclast resorption activity was reduced, but not compared to the scrambled control

A. Feline tooth resorption – Summary

Potential new therapeutic target – further studies required
Implications for osteoclast differentiation?

B. Feline Orals Squamous Cell Carcinomas –
Role of microenvironment in osteoclast activation

Feline Orals Squamous Cell Carcinomas (FOSCC)
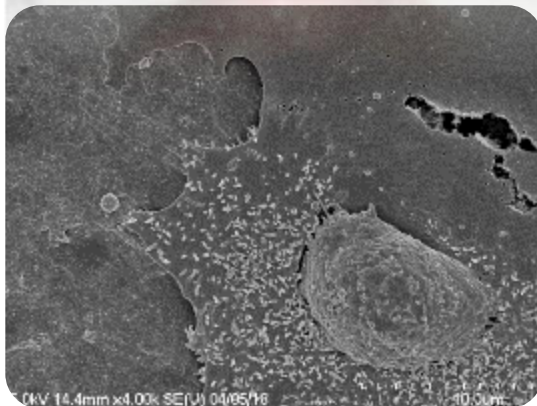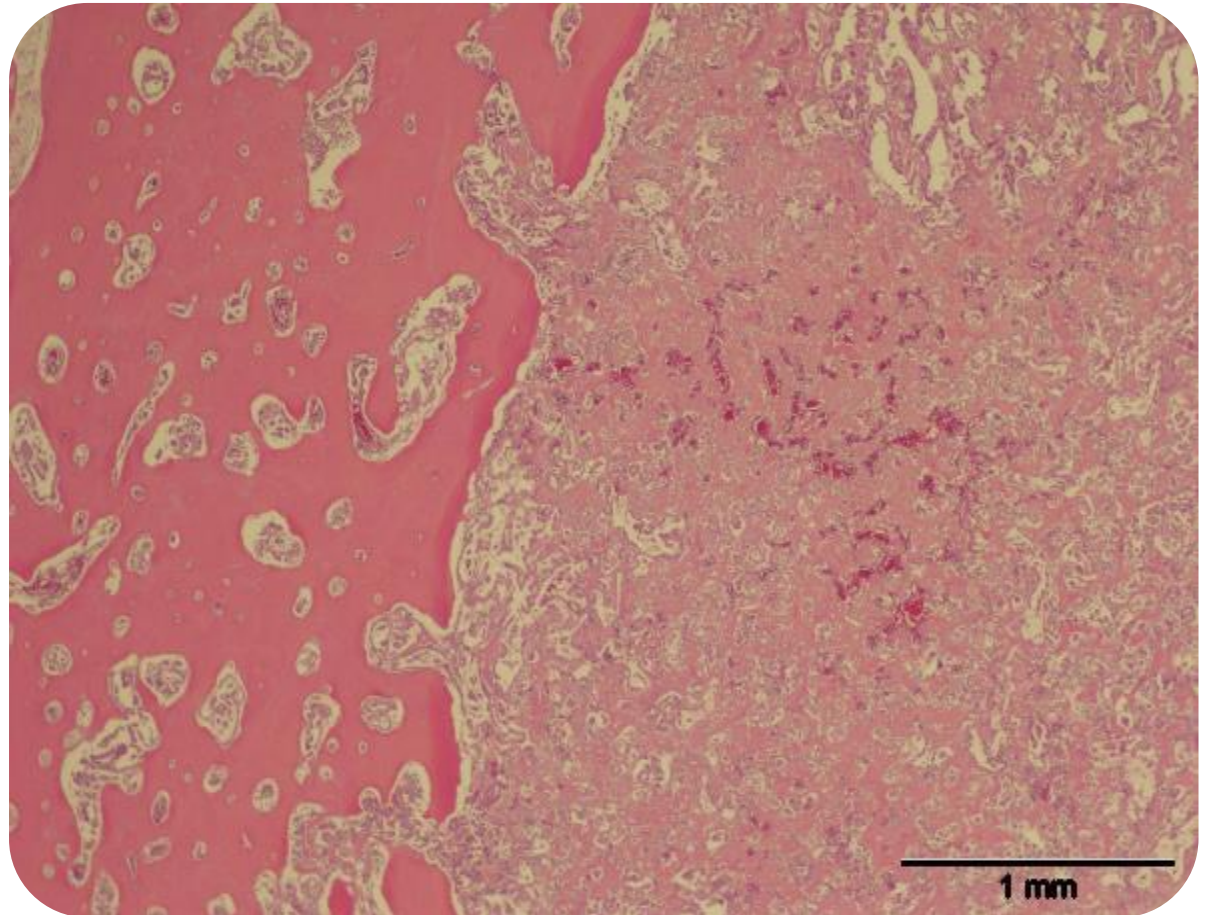
FOSCC invasion is mediated by osteoclasts
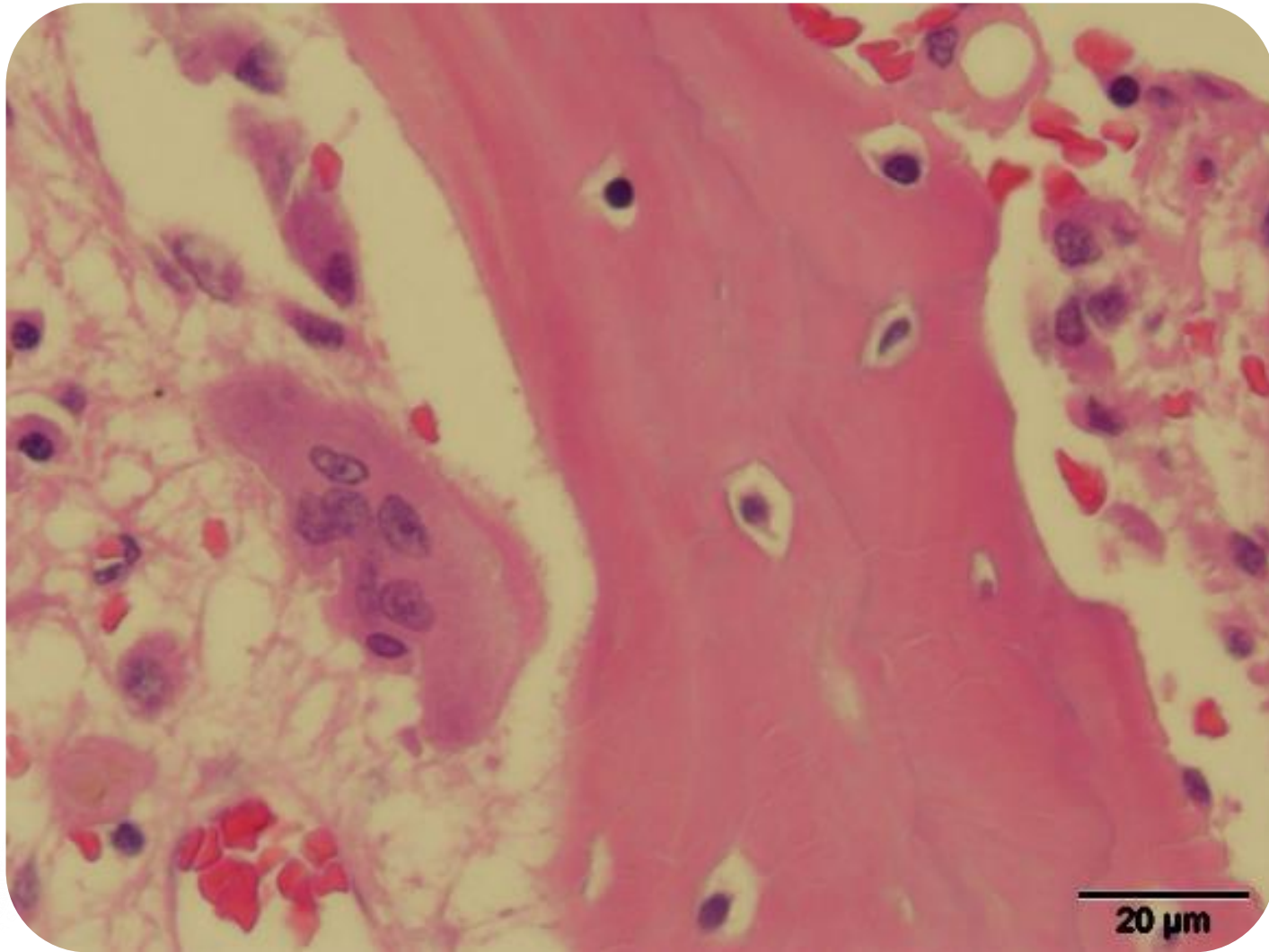
Role of microenvironment in osteoclast activation
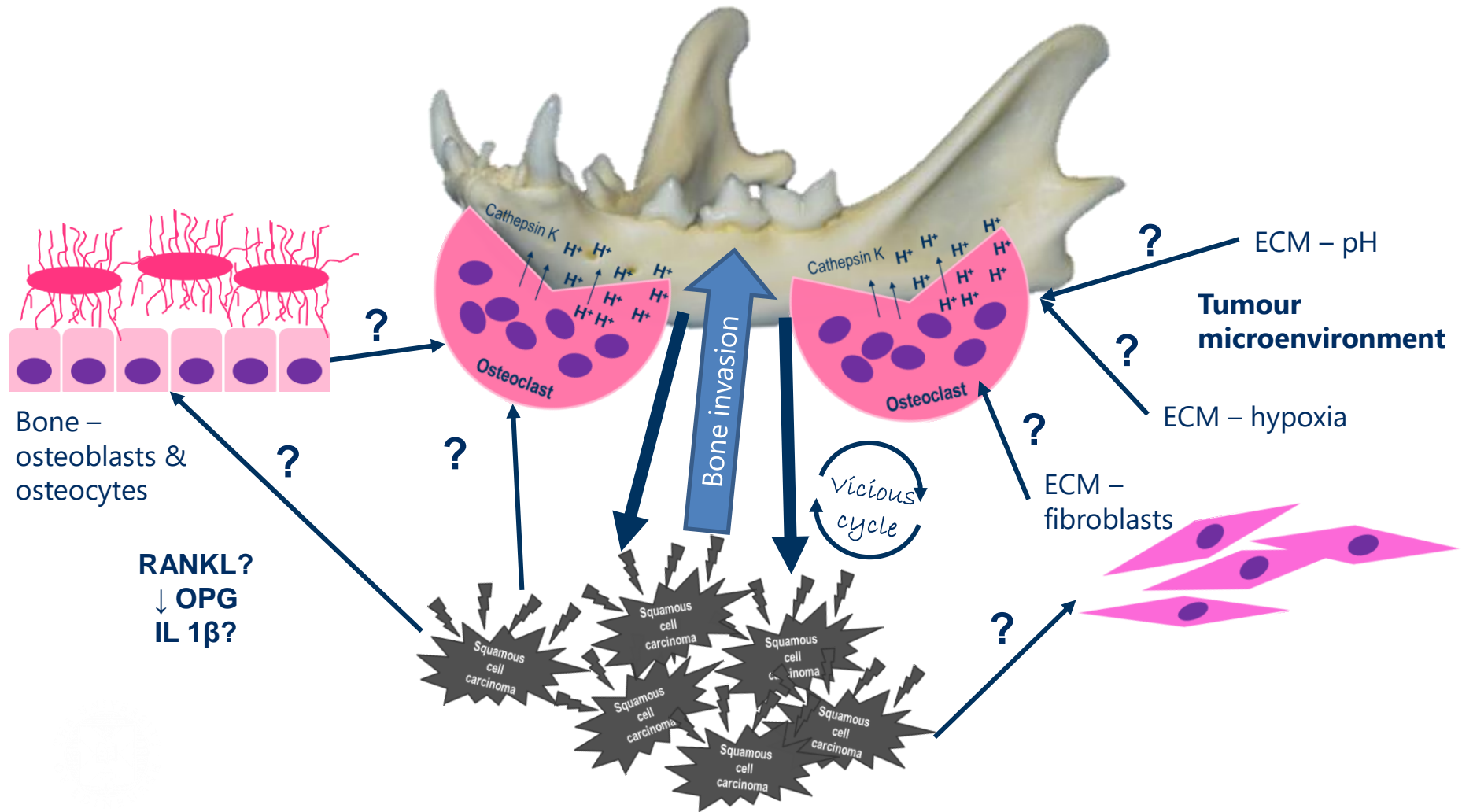
Proteomic analysis of bone invasive FOSCC cell line

Silver stain

SMG Conditioned medium protein precipitate

- Mass spectrophotometry revealed 168 different proteins (secreted, structural, enzymes)

- Identification of alternative/enhancing pathways to osteoclastogenesis

- Identification of possible cancer biomarkers

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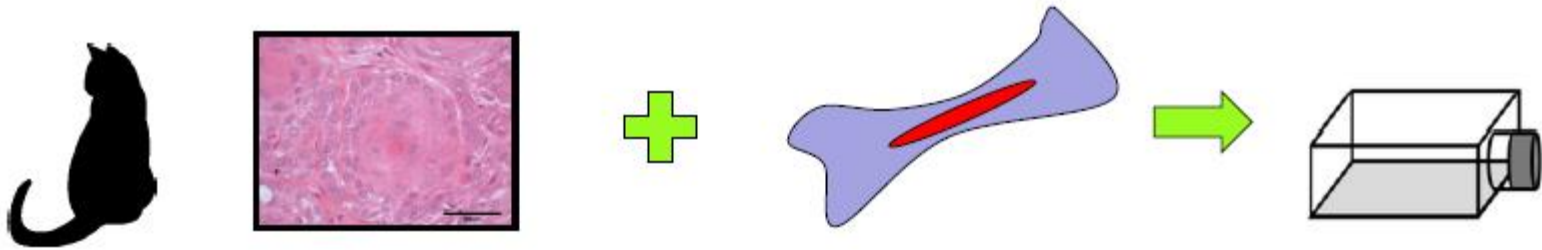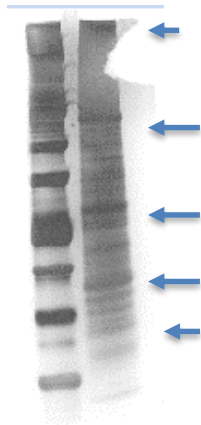Royal (Dick) School of Veterinary Studies

BBSRC
bioscience for the future

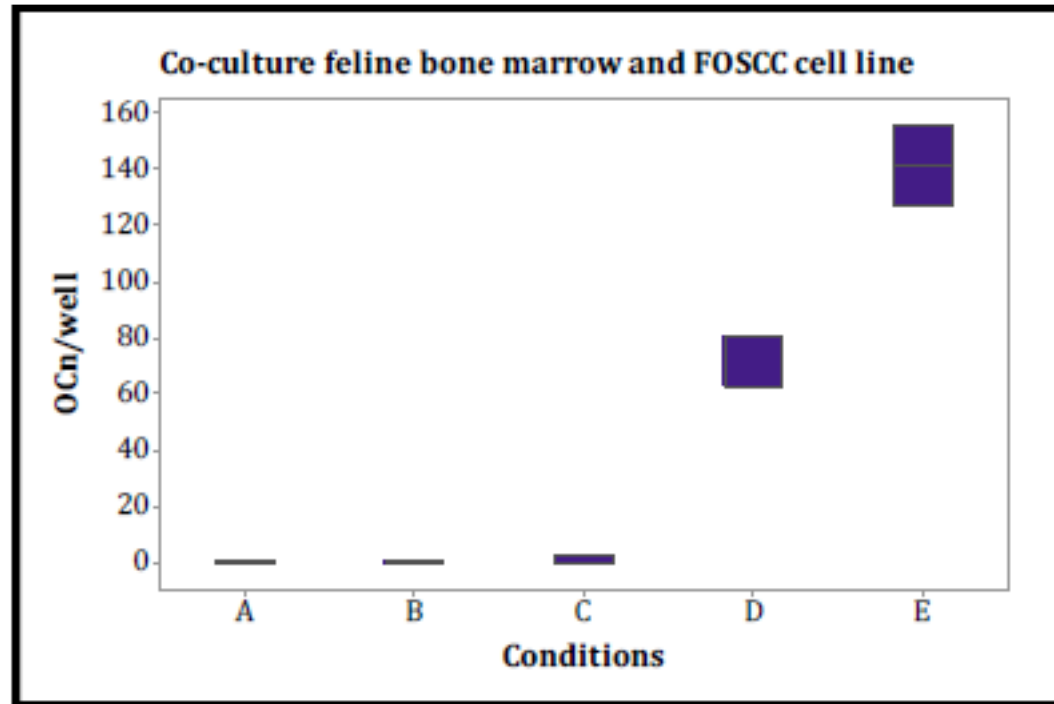# The effect of conditioned media on osteoclast formation and resorption

A – media only

B – media and conditioned media

C – CSF added

D – RANKL and CSF

E – RANKL, CSF & conditioned media

Co-culture feline bone marrow and FOSCC cell line

The effect of conditioned media on osteoclast formation and resorption

The presence of conditioned media from the bone invasive FOSCC cell line causes:

- Increased osteoclast formation

- Increased resorption on dentin and mineralised plates

- Increased survival of osteoclasts in hypoxic conditions
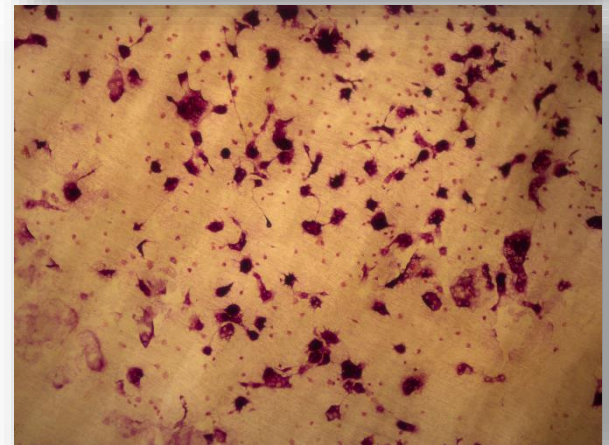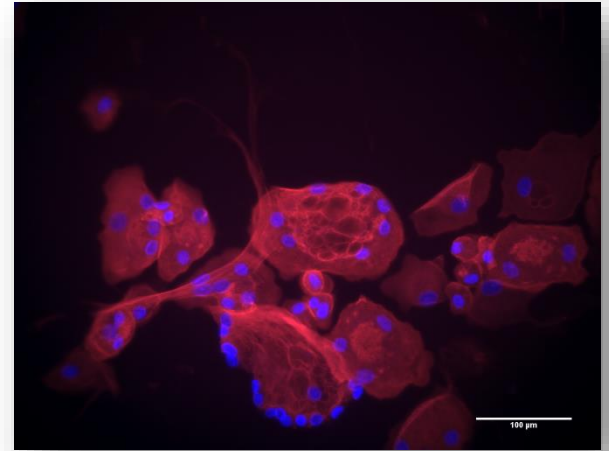
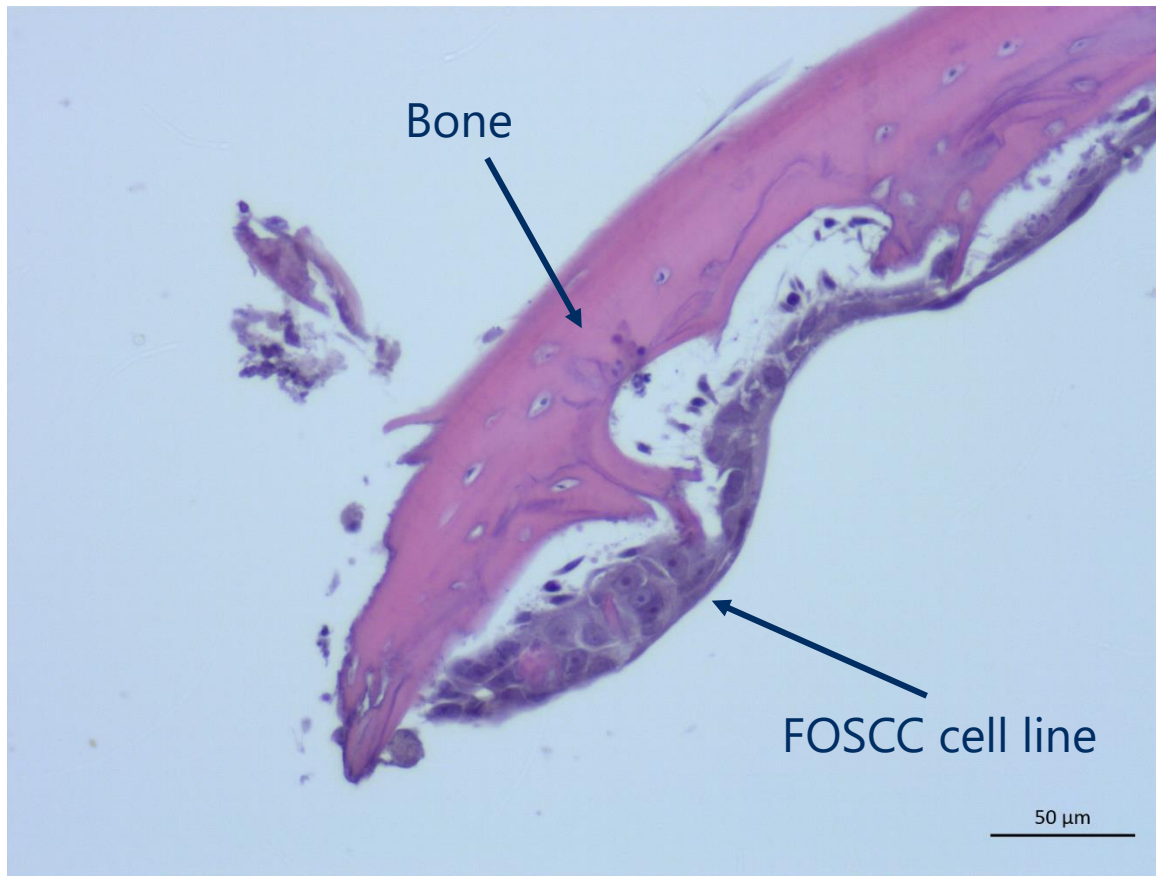# *Ex vivo* mouse calvaria co-culture experiment
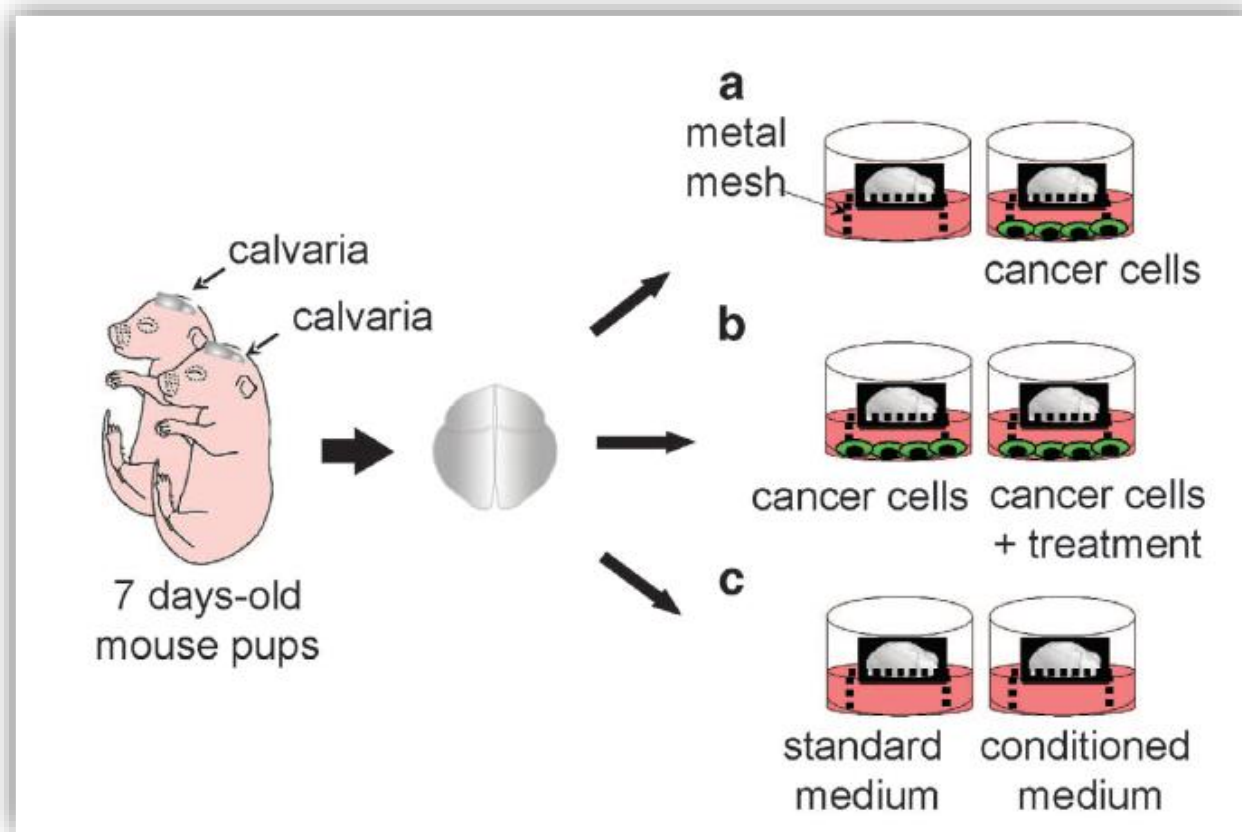
Ex vivo mouse calvaria co-culture experiment
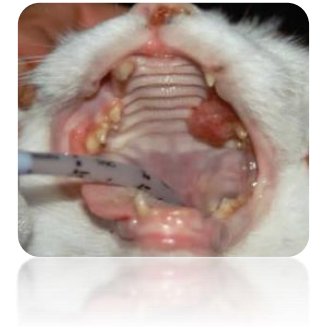
B. FOSCCs, Microenvironment & Osteoclasts – Summary

FOSCCs release substances that enhance osteoclast formation, resorption and survival

Proteomic analysis of CM from bone invasive and non-bone invasive tumours to narrow down potential candidates

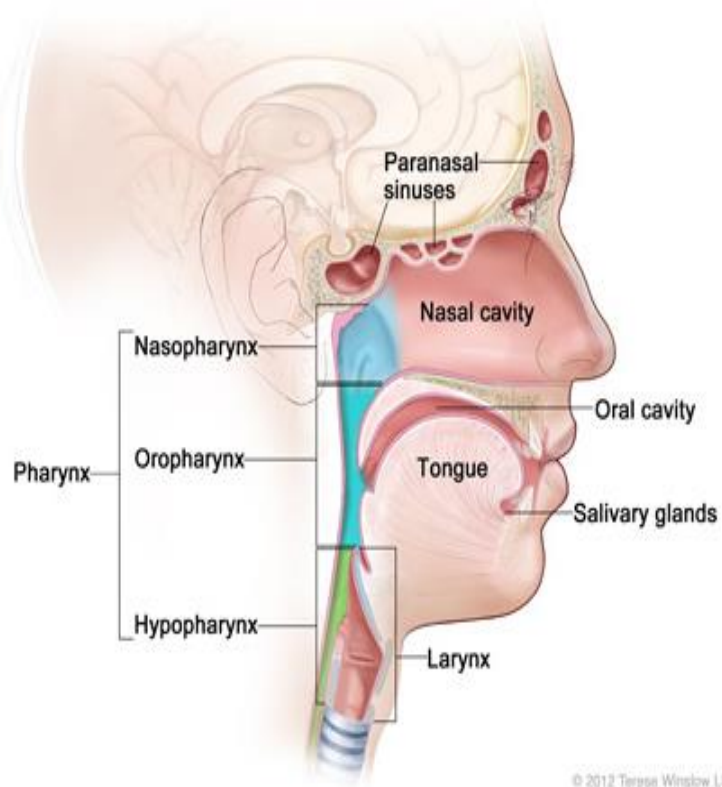# C. FOSCC –
Development of targeted therapies

HNSCC & FOSCC

Head and Neck Cancer Regions

Some common molecular markers:
- Epidermal Growth Factor Receptor (EGFR)
- c-Src

Targeted therapies using RNA interference – siRNA validation
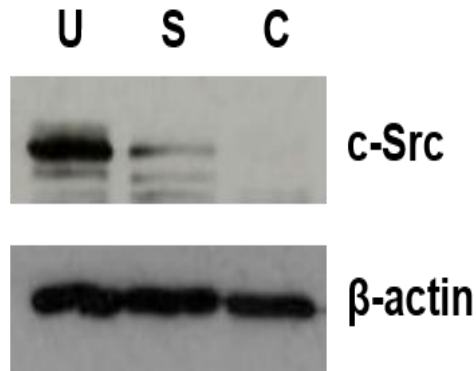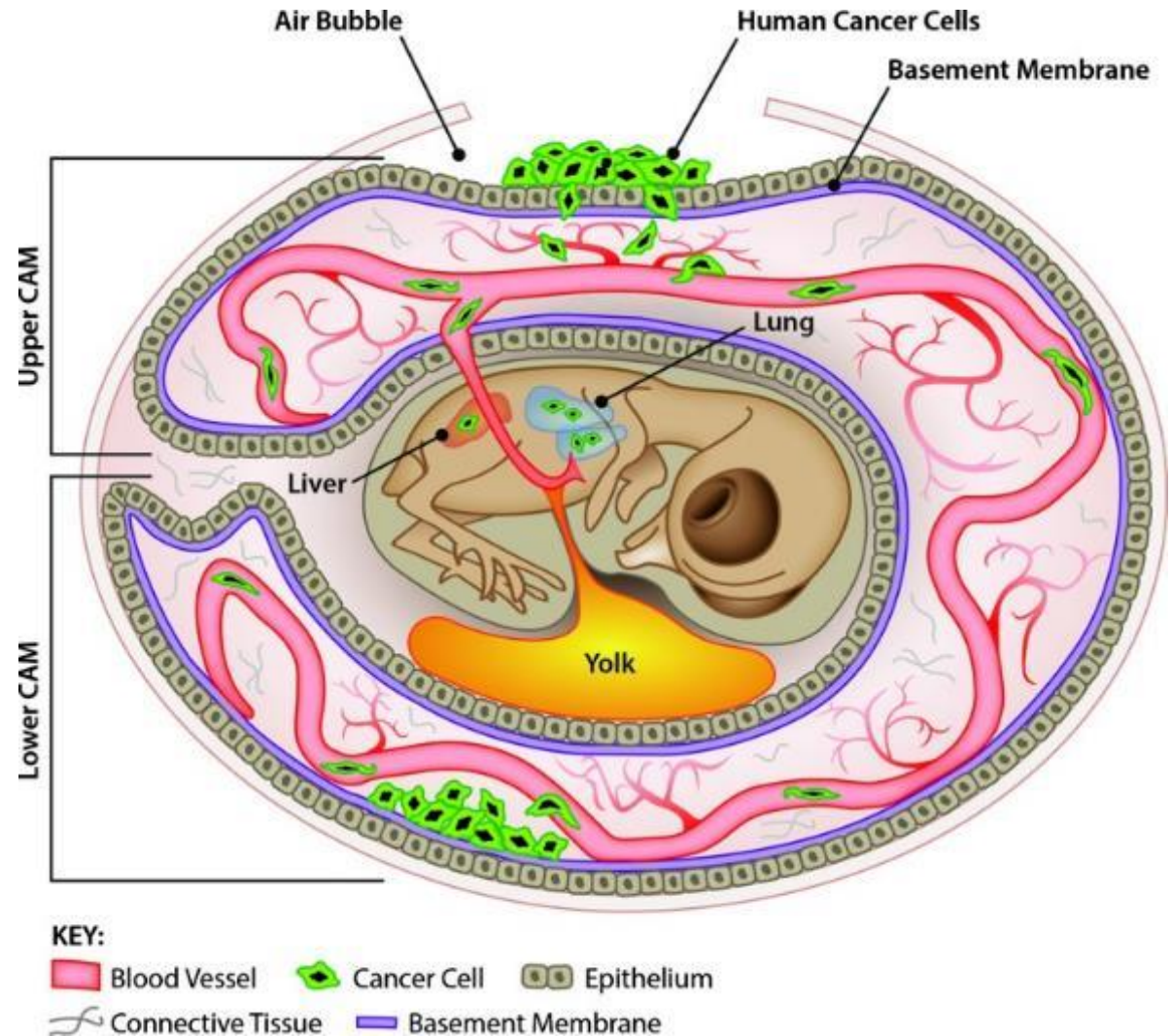

c-Src siRNA

EGFR siRNA
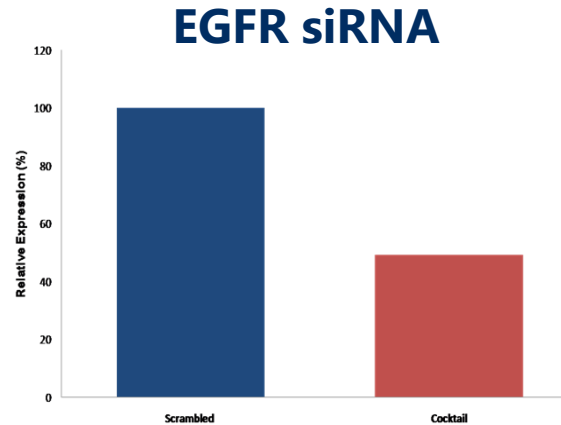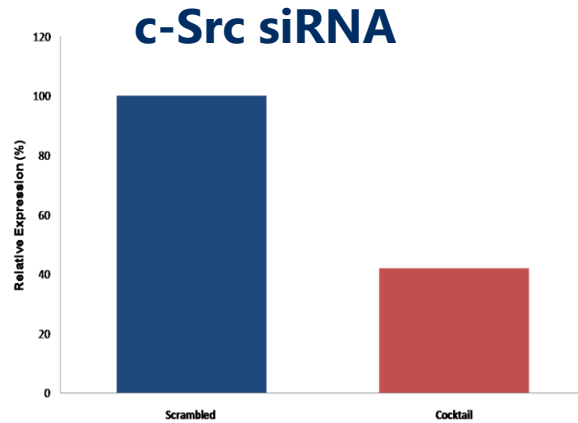
qPCR – relative mRNA levels
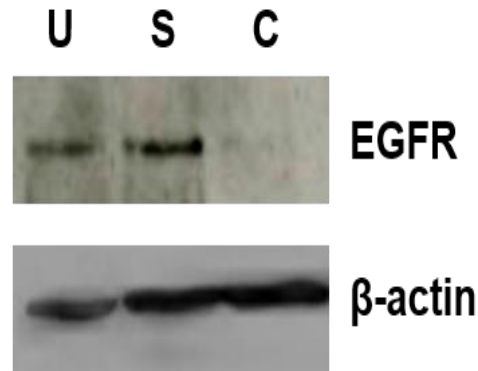
Western Blot – relative protein levels

U: Untreated
S: Scrambled
C: Cocktail

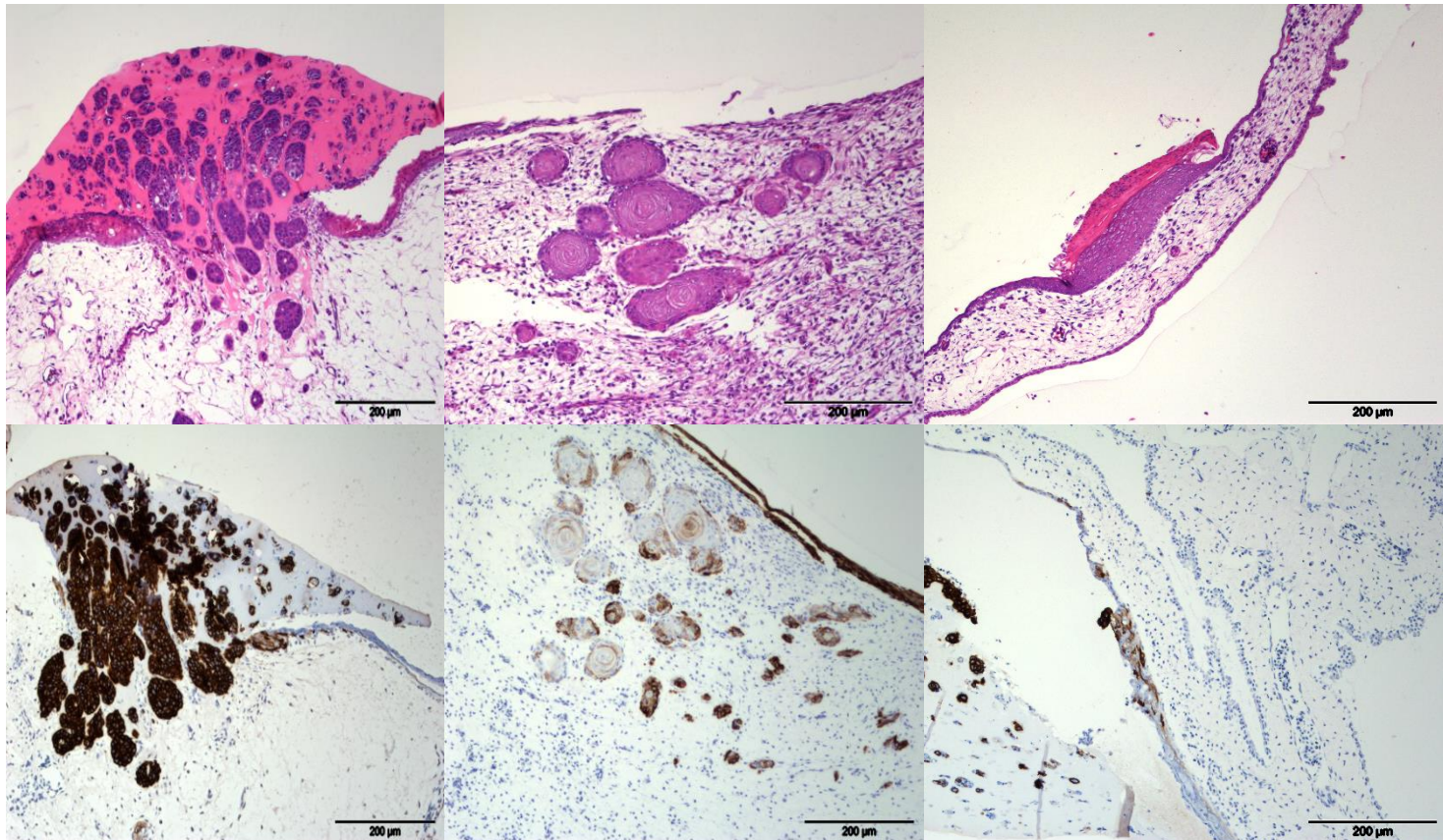# Chicken Chorioallantoic Membrane (CAM) model

Liu M, Scanlon CS, Banerjee R, Russo N, Inglehart RC, Willis AL, et al. Translational Oncology. 2013;6(3):273-81.

Development of targeted therapies

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Thank you for you attention