Flame Retardants and Environmentally Relevant Mixtures Induce Adipogenesis: What are the Long-Term Impacts on Metabolic Disorders?

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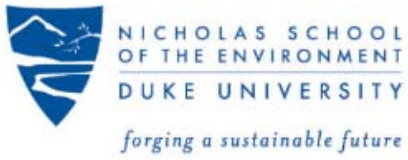


Developmental Origins of Health And Disease (DOHAD)

www.dohadfordoctors.com

- Strong link between perinatal and early childhood factors and risk of chronic disease (e.g. heart disease, diabetes, obesity, etc)
- But what chemical exposures are most important to research?

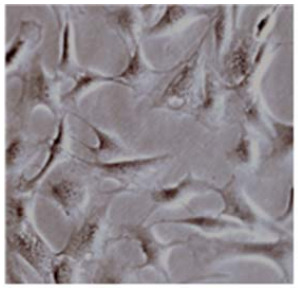
Research Questions?

- How do halogenated and organophosphate based organic contaminants affect:
  - Thyroid hormone regulation
  - Adipogenesis
  - Osteogenesis

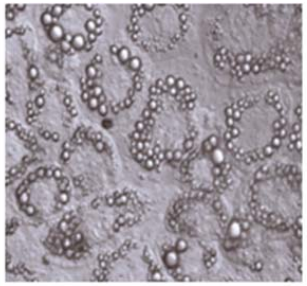
- Will exposure to environmentally relevant mixtures (e.g. house dust) impact these pathways?

Adipogenesis Assay Measures

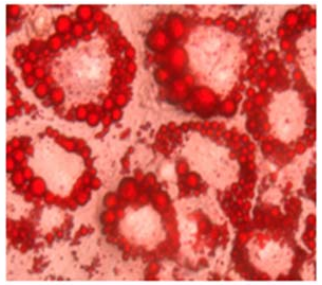
- Triglyceride accumulation
  - AdipoRed - hydrophilic fluorescent dye (Nile Red)
    - Partitions into lipid droplets in the cells, fluoresces



(A)



(B)



(C)

- Cell proliferation/cytotoxicity
  - NucBlue DNA dye (Hoechst 33342)
    - Partitions into nuclei and fluoresces upon binding DNA

Adipogenesis Assay Me

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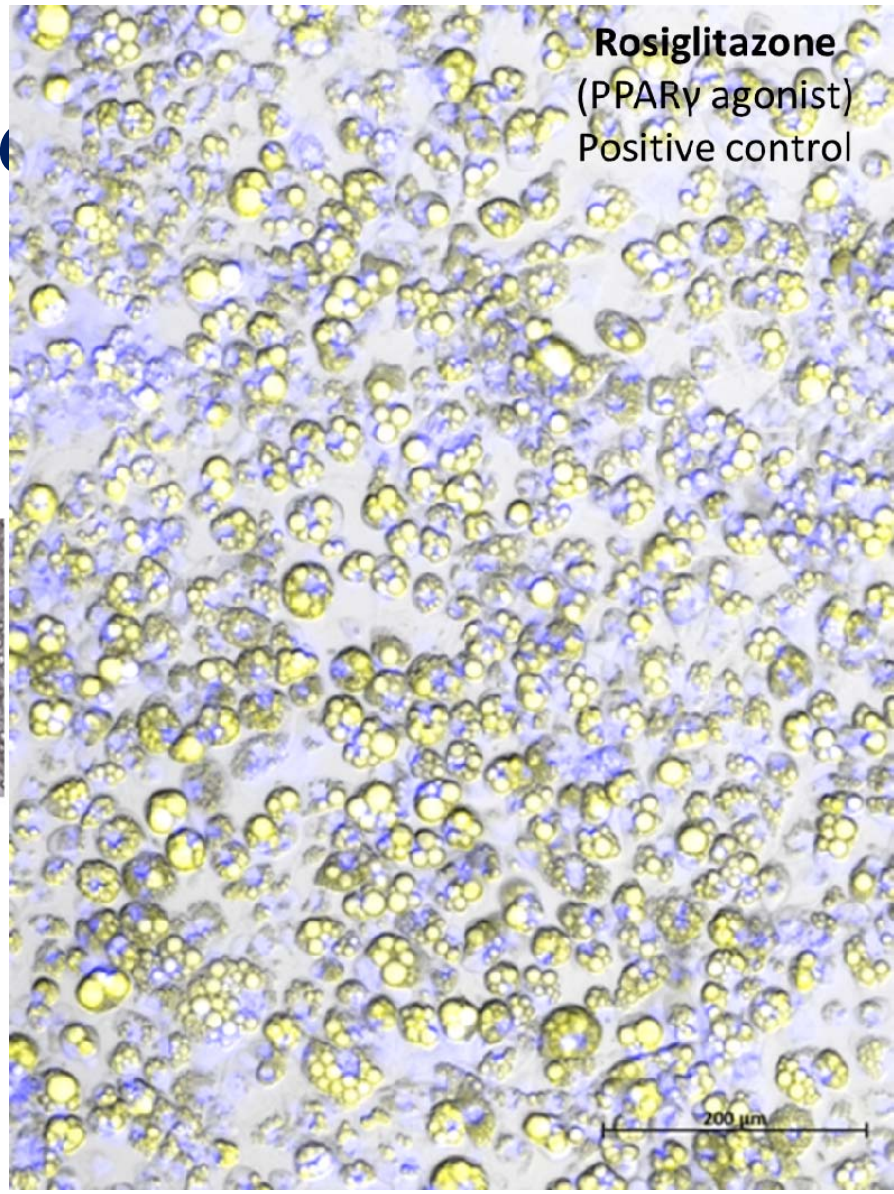
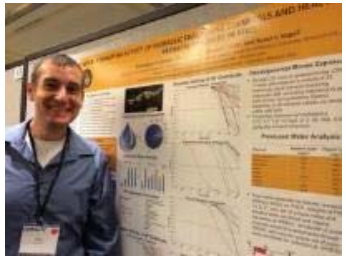
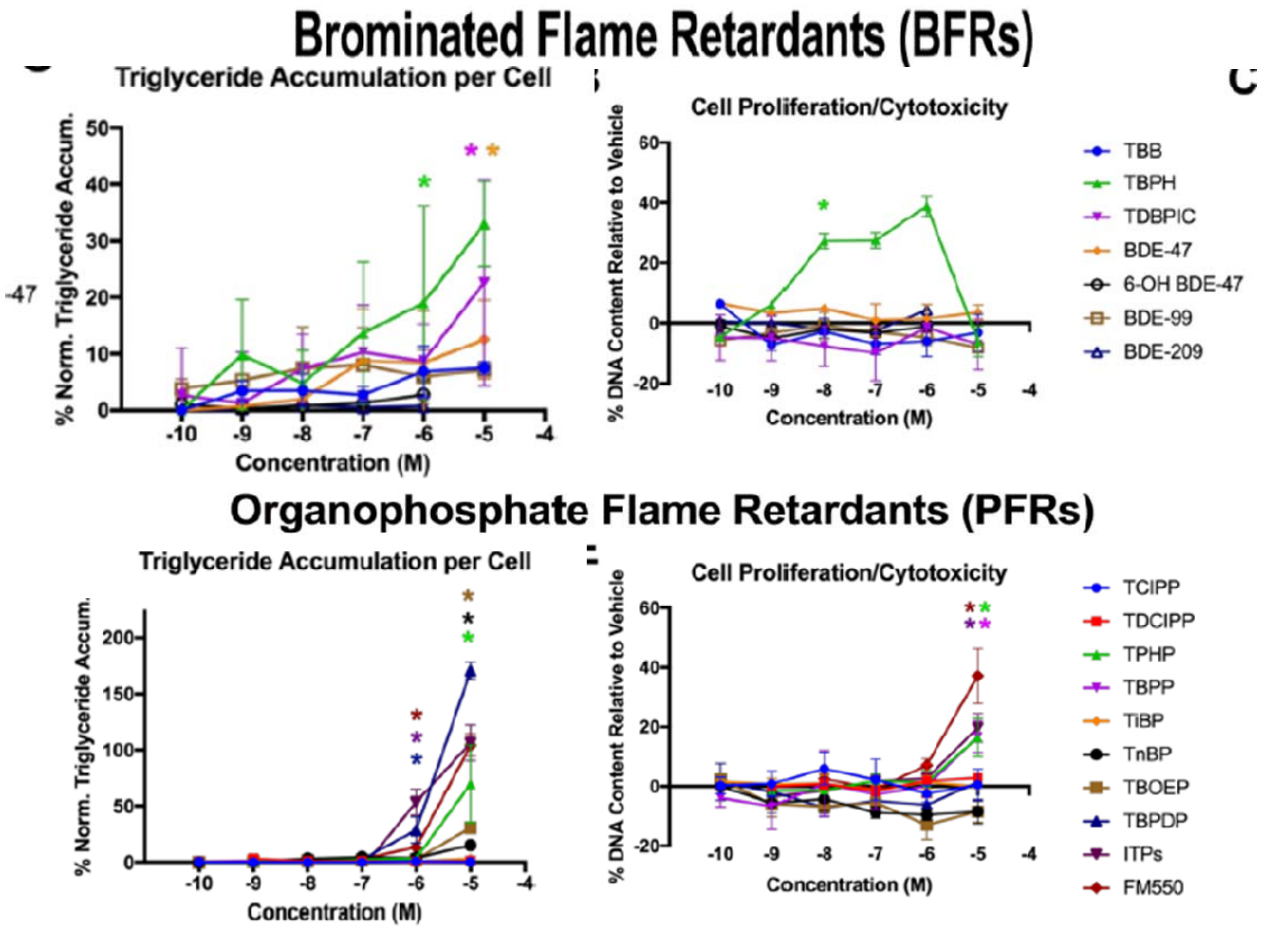


Flame Retardants and Adipogenesis

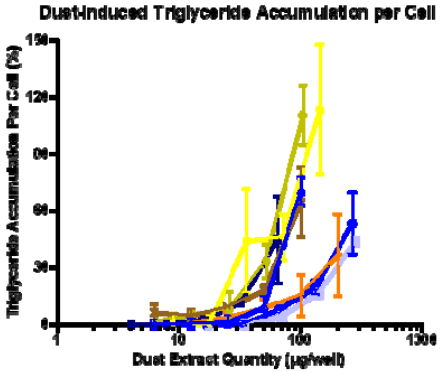


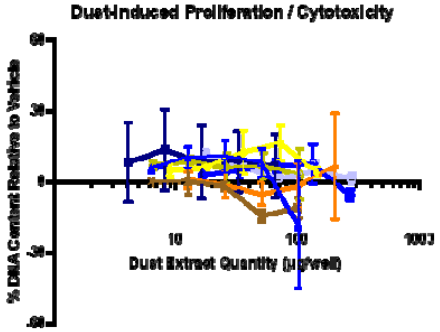
Chris Kassotis, PhD



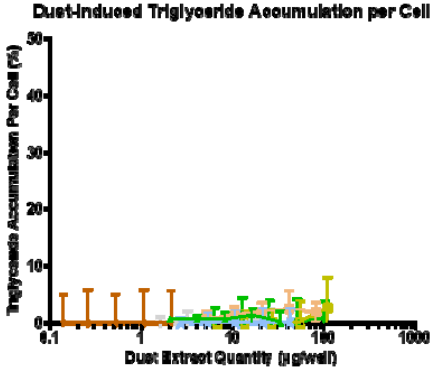
Kassotis et al. 2017, *ES&T*

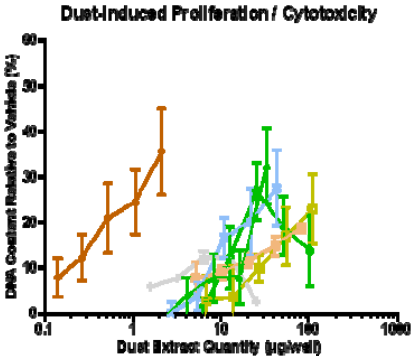
Adipogenesis "Phenotypes" Observed in Dust Extracts





- **High triglyceride accumulation**
- **Minimal pre-adipocyte proliferation**





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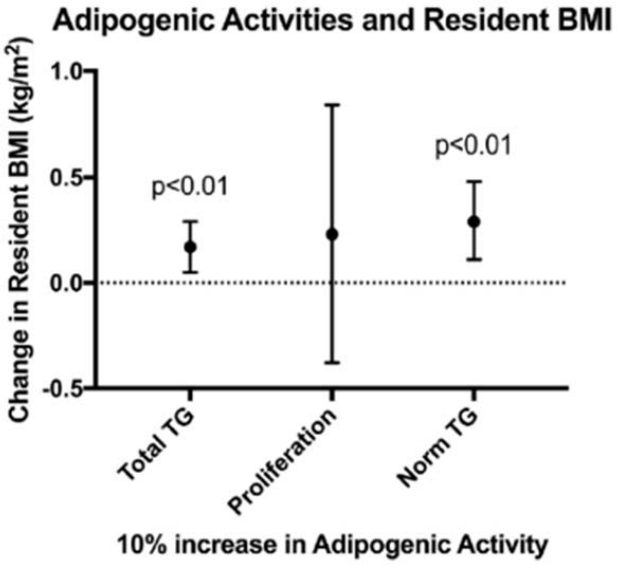
Kassotis et al. 2019, *STOTEN*

Health Outcomes and Adipogenic Activities of House Dust

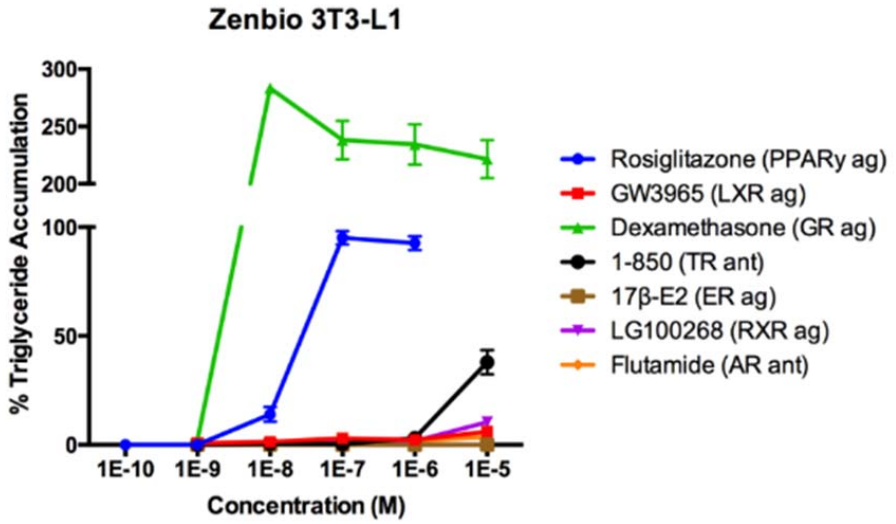


- Performed regressions controlling for sex, age, race, and education as potential confounders.
  - Using both efficacy and potency of triglyceride accumulation/ proliferation metrics
- Triglyceride accumulation efficacy was significantly associated with resident BMI.

Kassotis et al. 2019, *STOTEN*

Putative Role of Thyroid Receptor β Antagonism in Adipogenic Activity

- GR (dexamethasone) and PPARγ (rosiglitazone) are potent and efficacious regulators of adipogenesis.
- 1-850 (non-specific TRβ isoform antagonist) also significantly promotes adipocyte differentiation.
- Triglyceride accumulation (3T3-L1 cells) significantly correlated with TRβ antagonism in dust extracts (r_s = 0.447; p<0.001).
  - Not correlated with pre-adipocyte proliferation

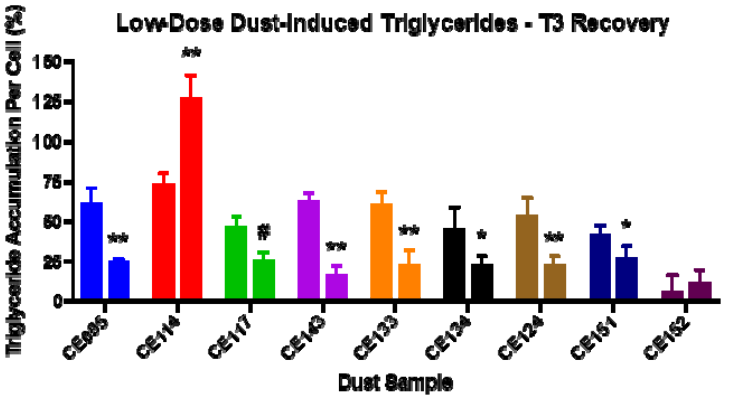


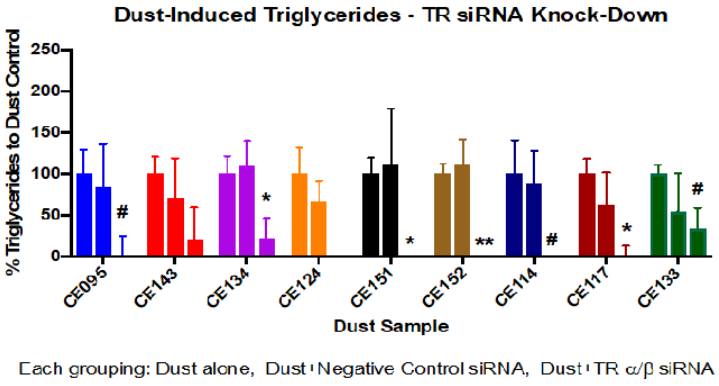
Kassotis et al. 2017, *Sci Rep*

Kassotis et al. 2019, *STOTEN*

Contributory Role of TRβ Antagonism in Adipogenic Activity

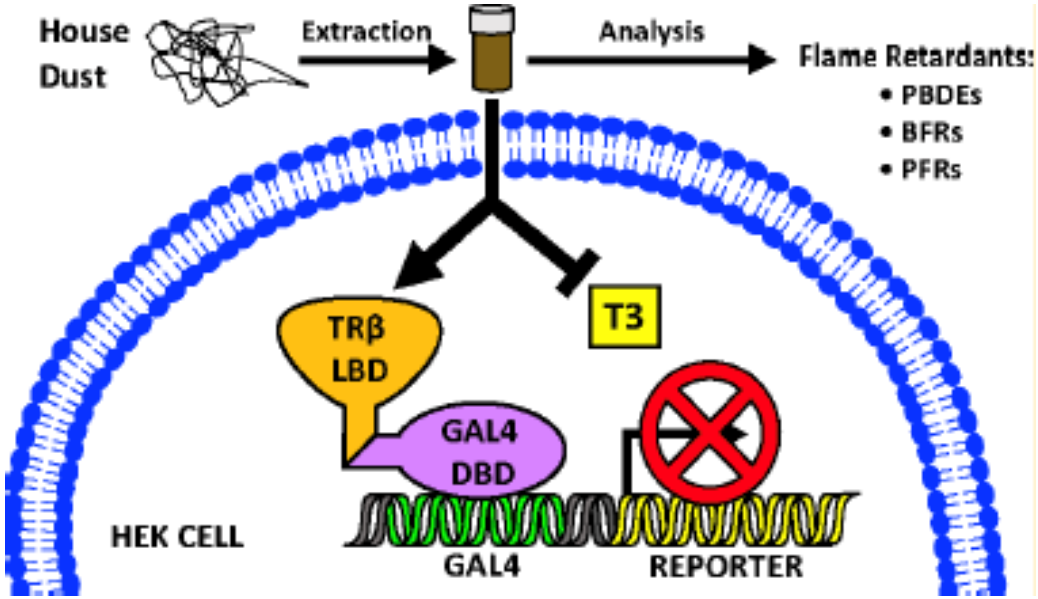
- Two experiments bolster causative link between TRβ and triglyceride accumulation in 3T3-L1 cells:
  - Ligand recovery experiment. Dust + T3 (TR agonist):
    - Addition of T3 inhibited dust-induced triglyceride accumulation for 7 of 9 samples.
  - siRNA knock-down of TRα/β:
    - TR knock-down inhibited dust-induced triglyceride accumulation for 7 of 9 samples (two trending).





Kassotis et al. 2019, *STOTEN*

Are Chemical Mixtures in House Dust "Active"?



Kollitz et al. 2018, *ES&T*

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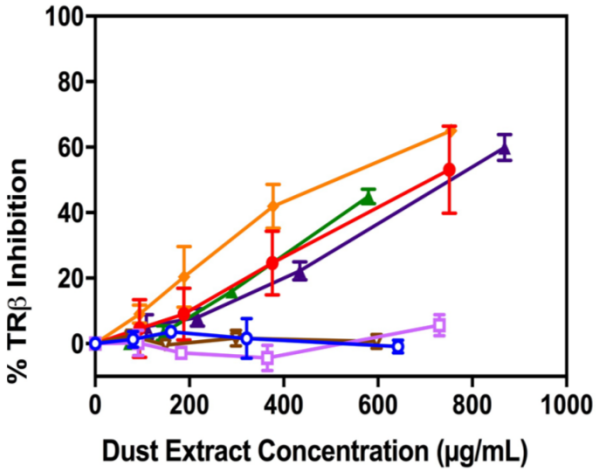


Figure 1. Representative results of TRβ antagonism (% inhibition) by active (closed shapes) and inactive (open shapes) dust extracts obtained via the GeneBLAzer β-lactamase reporter assay in HEK 293T cells as described in the Materials and Methods. The colors represent different dust extracts. Cells were treated with a range of dust extract concentrations in the presence of 0.3 nM triiodothyronine (T3). Extracts that decreased TRβ activity $\geq$20% of the T3 control were considered active. Plotted data is the average $\pm$ SEM of three separate experiments.

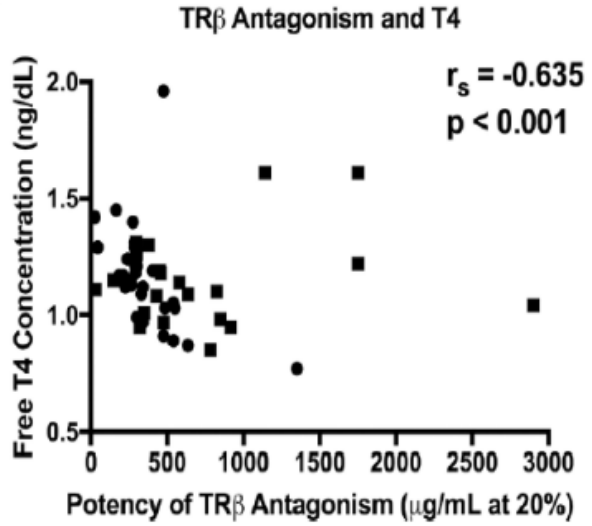


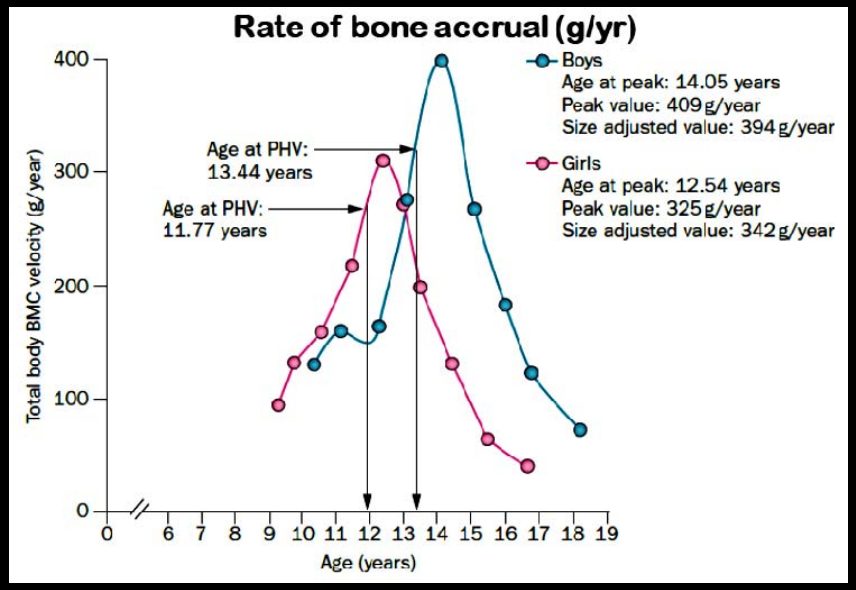
Figure 4B. Spearman correlation between TRb antagonism of house dust vs serum T4 levels of residents

Kollitz et al. 2018, *ES&T*

What about osteogenesis?

Is There a Developmental Basis of Adult Bone Disease?

- Peak bone mass achieved during childhood/puberty
- Important determinants of bone mass and fracture risk later in life
- May contribute to adult degenerative bone diseases



Farr and Khosla, *Nat Rev Endocrinol*, 2015

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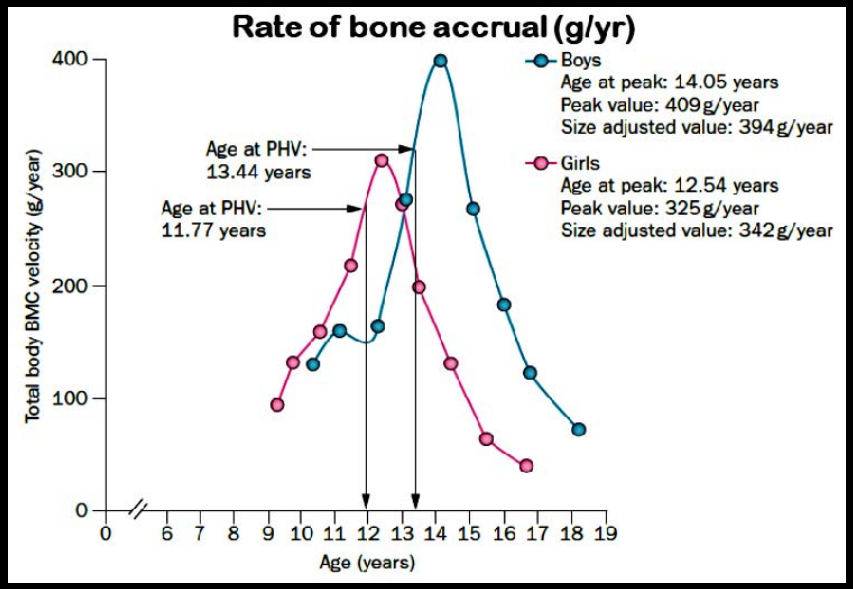


Farr and Khosla, *Nat Rev Endocrinol*, 2015

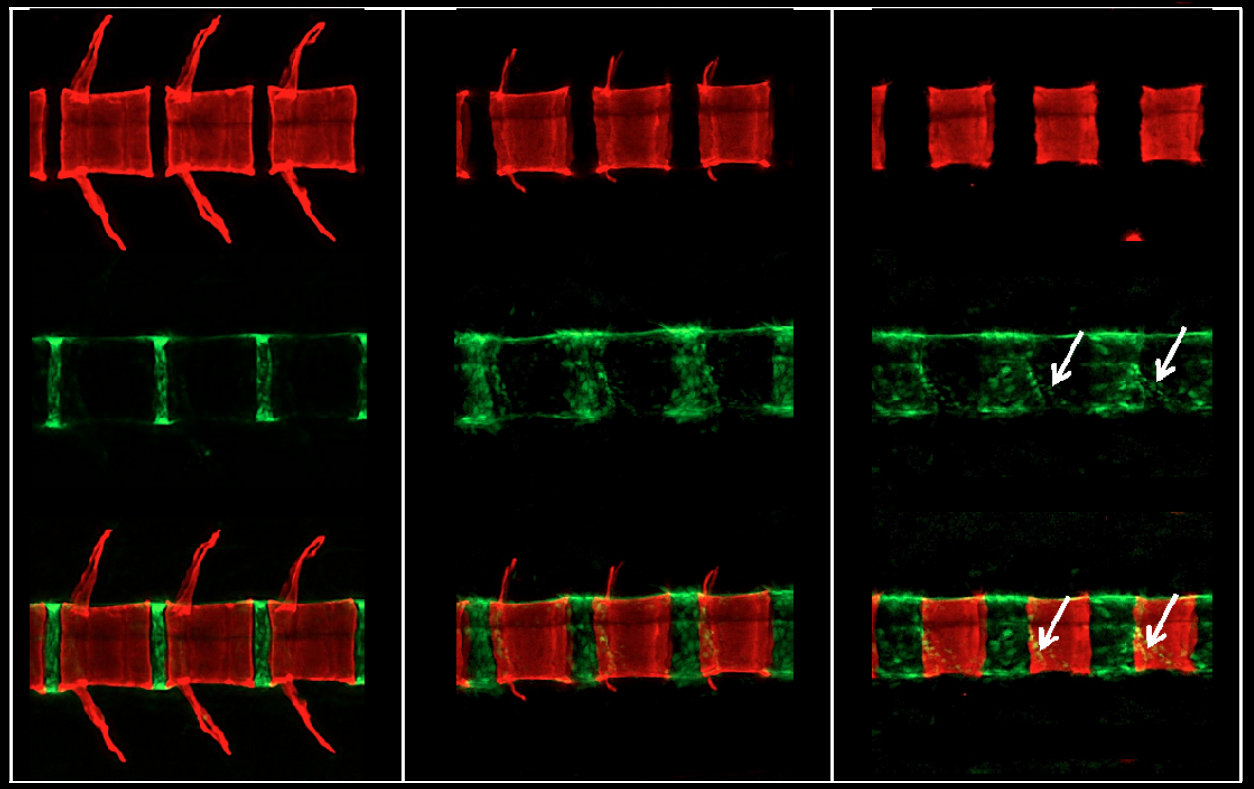
Can Environmental Exposures Contribute to Osteochondral-Dysplasia & Degenerative Disease?

TCDD induced osteochondral phenotypes

TG (Twist-GFP): MSC

Alizarin Complexone

4 hr exp, 20 dpf



TCDD Recapitulates human skeletal disease

Categories	Disease or Function	p-value	# Genes
Connective Tissue Disorders, Skeletal & Muscular Disorders	Arthropathy	4.73E-04	51
Connective Tissue Disorders, Inflammatory Disease, Skeletal & Muscular Disorders	arthritis	9.78E-04	49
Cell Morphology, Cellular Function & Maintenance	autophagy of cells	1.13E-05	20
Protein Degradation, Protein Synthesis	degradation of protein	1.19E-09	45
Metabolic Disease	disorder of lipid metabolism	1.20E-03	14
Cell Death and Survival	cell death of connective tissue cells	1.77E-06	41
Organismal Injury & Abnormalities	fibrosis	6.44E-04	30
Skeletal & Muscular Disorders	myopathy	1.48E-07	40
Skeletal & Muscular System Development/Function	mineralization of bone	4.93E-04	12
Skeletal & Muscular System Development/Function	osteoclastogenesis	8.95E-05	10

Shared targets between congenital skeletal dysplasias and embryonic TCDD exposure.

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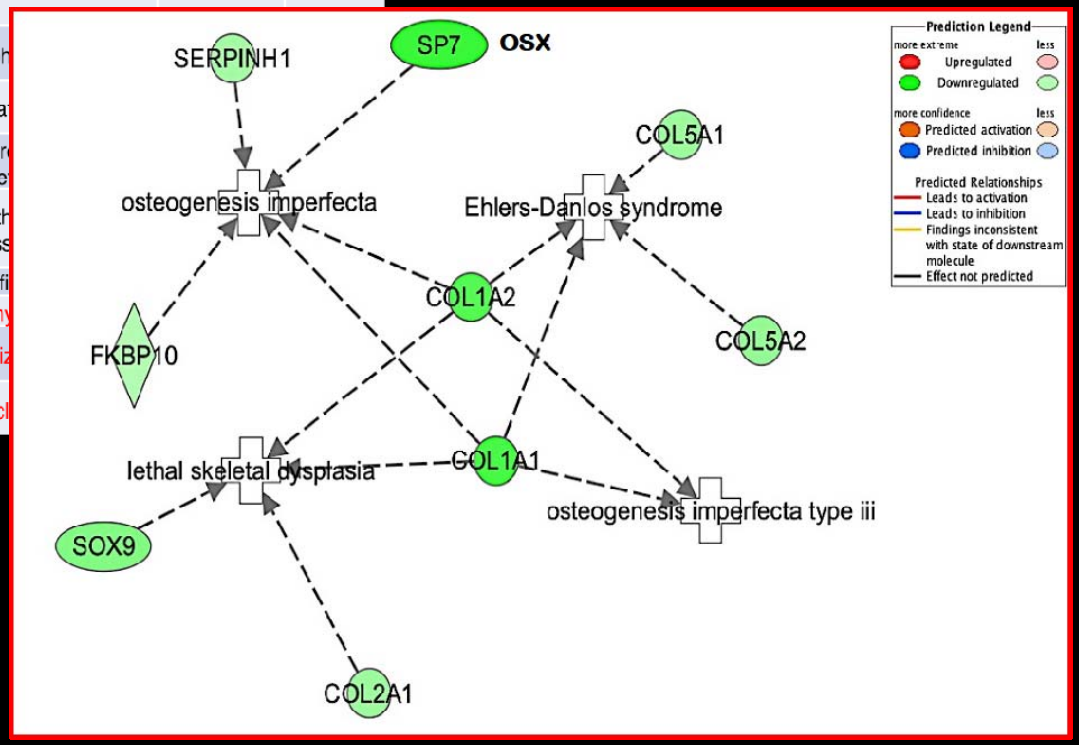
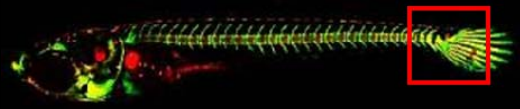


TPP& FM 550 Osteochondral Phenotypes

Tg(Col10a11-GFP)

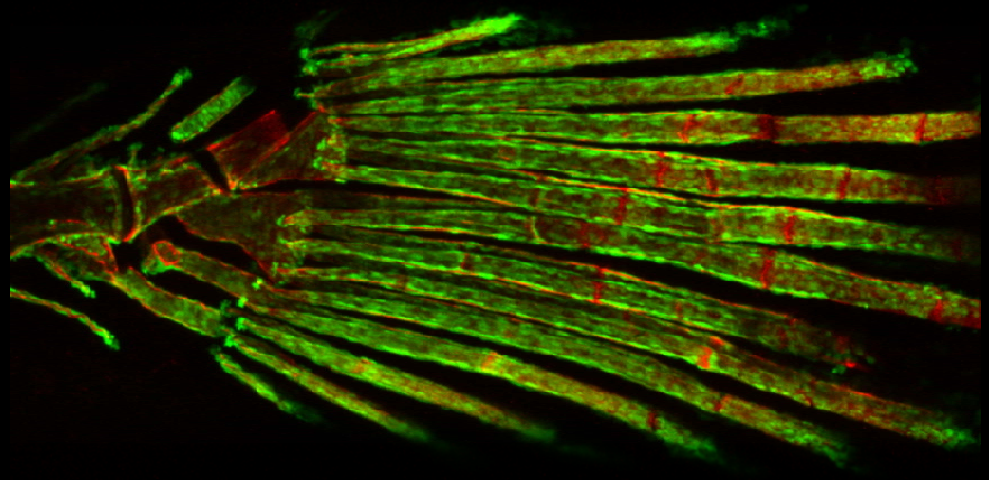
Alizarin Complexone

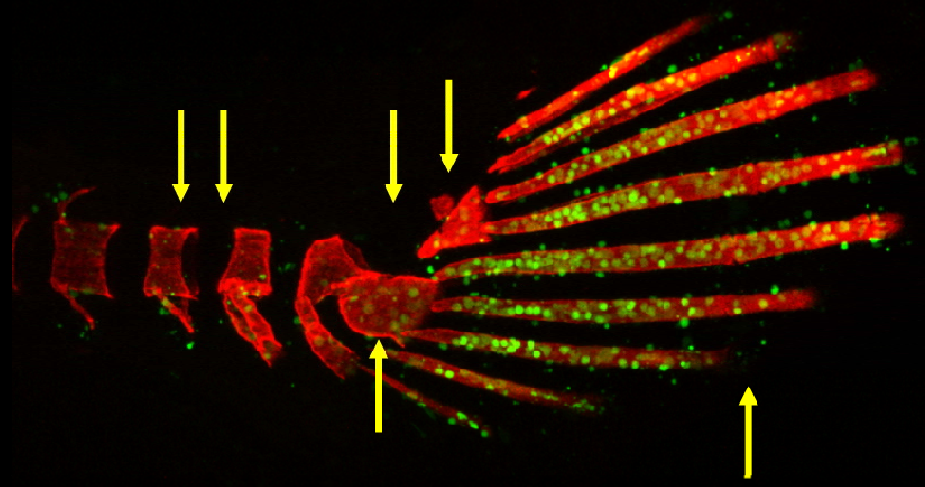


19d exp, 19 dpf

DMSO

FM 550 20 µM , TPP 0.75



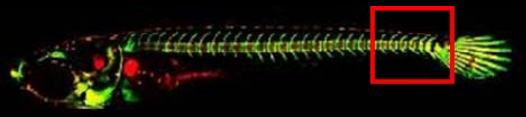


FM 550 treated medaka display increased IVR, deformed centra, reduced hypural cartilage decrease in col10a1 expression/localization throughout the caudal fin and axial spine

TPP & FM 550 Osteochondral Phenotypes

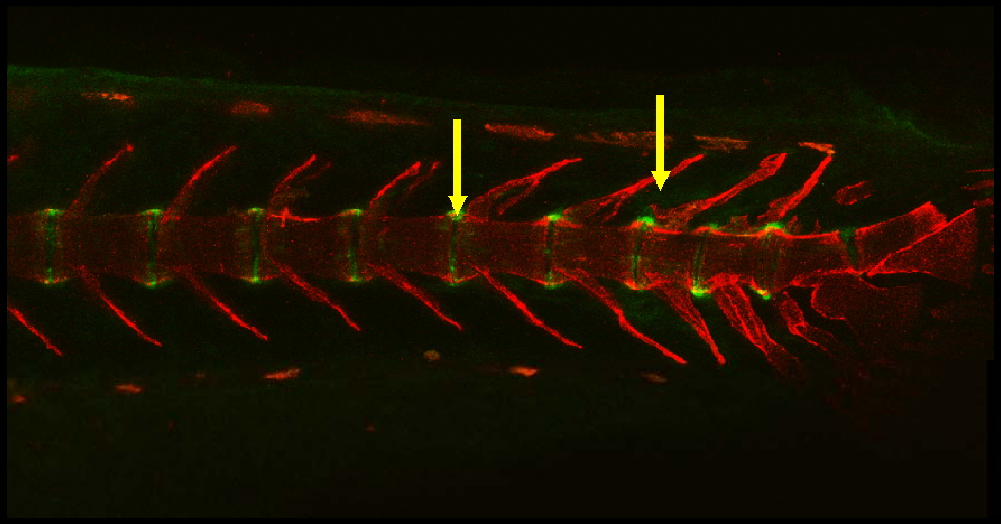
TG (Twist-GFP)

Alizarin Complexone

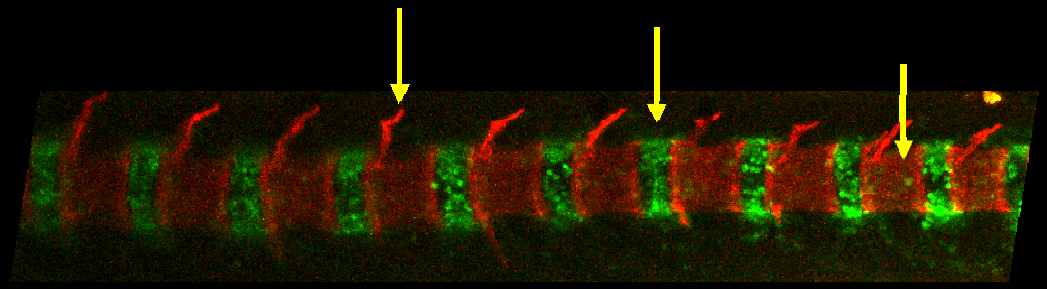


19d exp, 19 dpf

DMSO



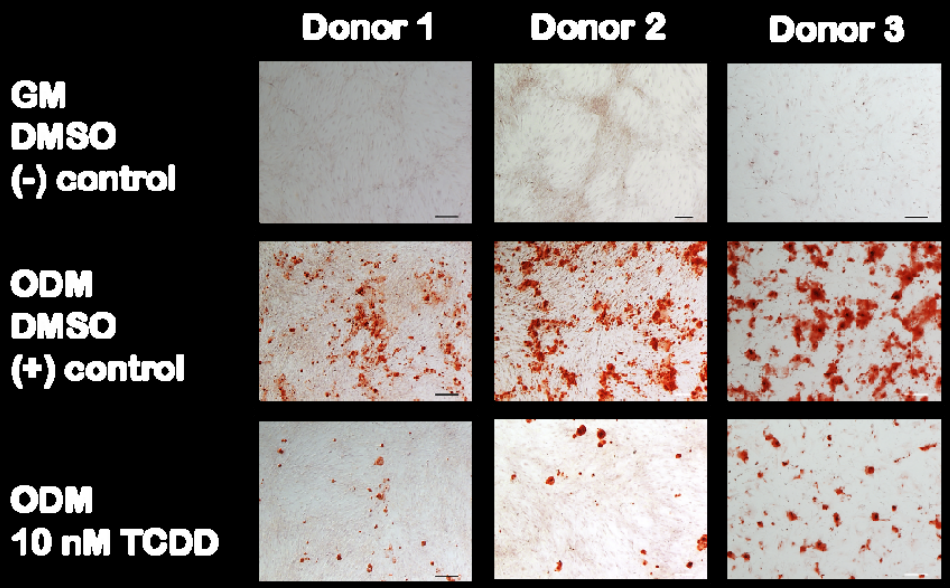
TPP 0.75 µM , FM 550 20 µM

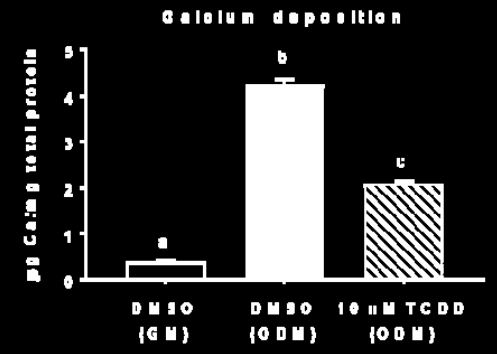


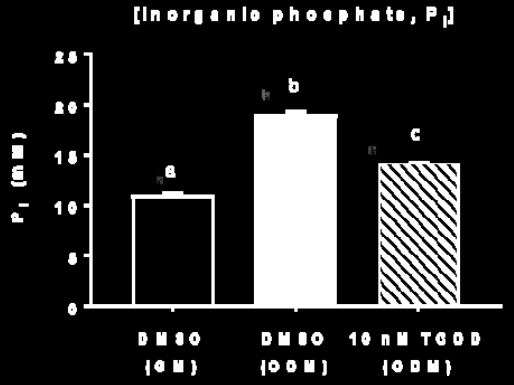
Treated medaka display increased IVR and displaced expression of twist+ cells within IVR, some evidence of twist + cells within the calcifie centrum

Human Mesenchymal Stem Cells

TCDD Induces Loss of Osteogenic Differentiation







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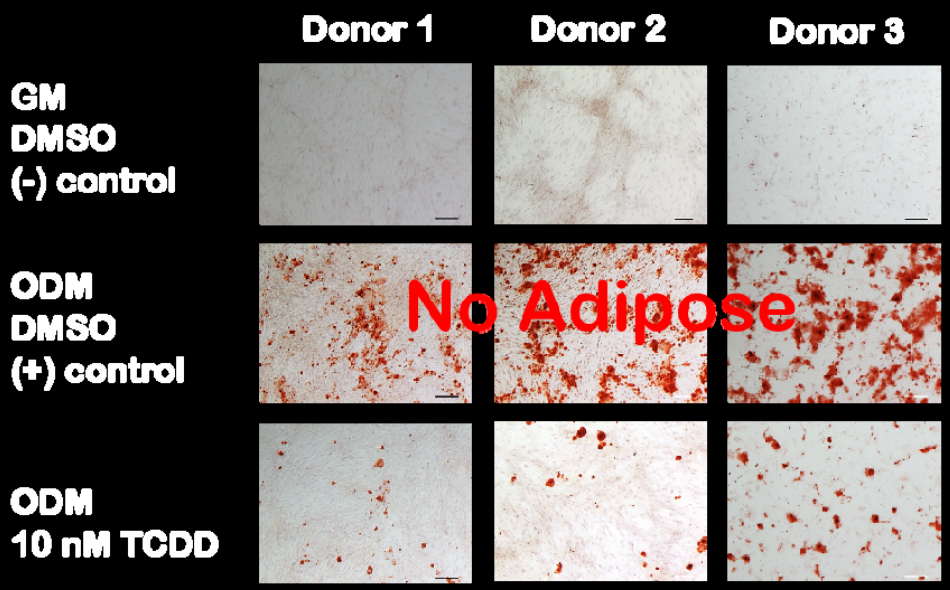
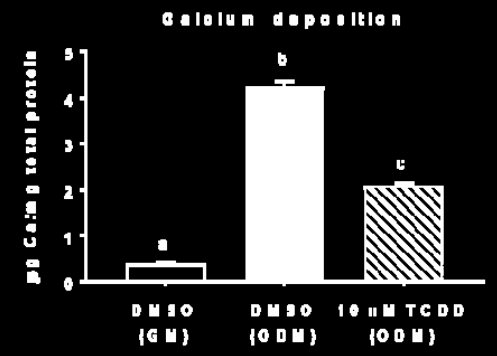
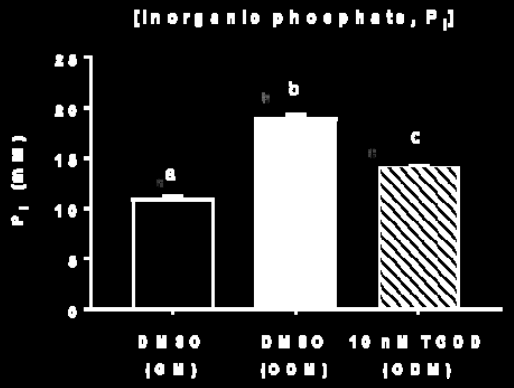
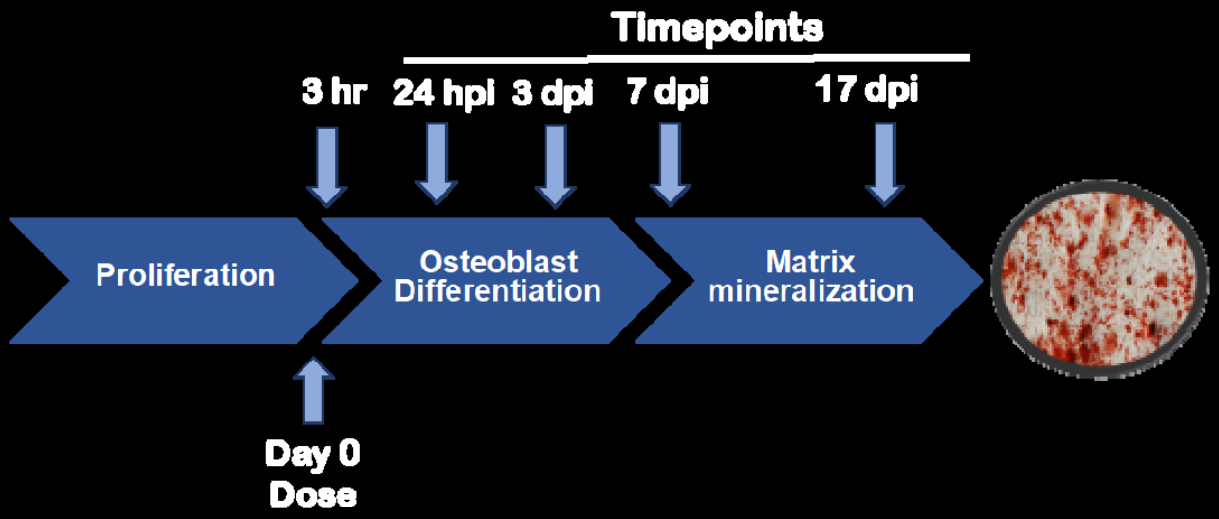
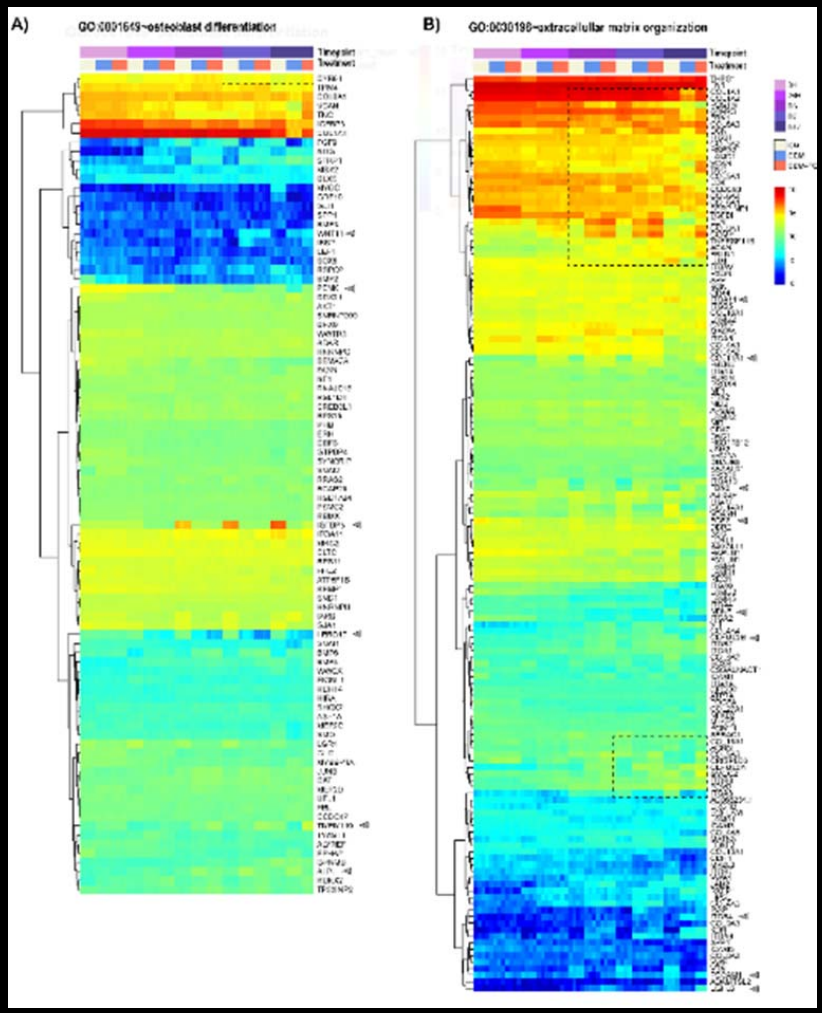






TCDD Modulates MSC Commitment/Differentiation





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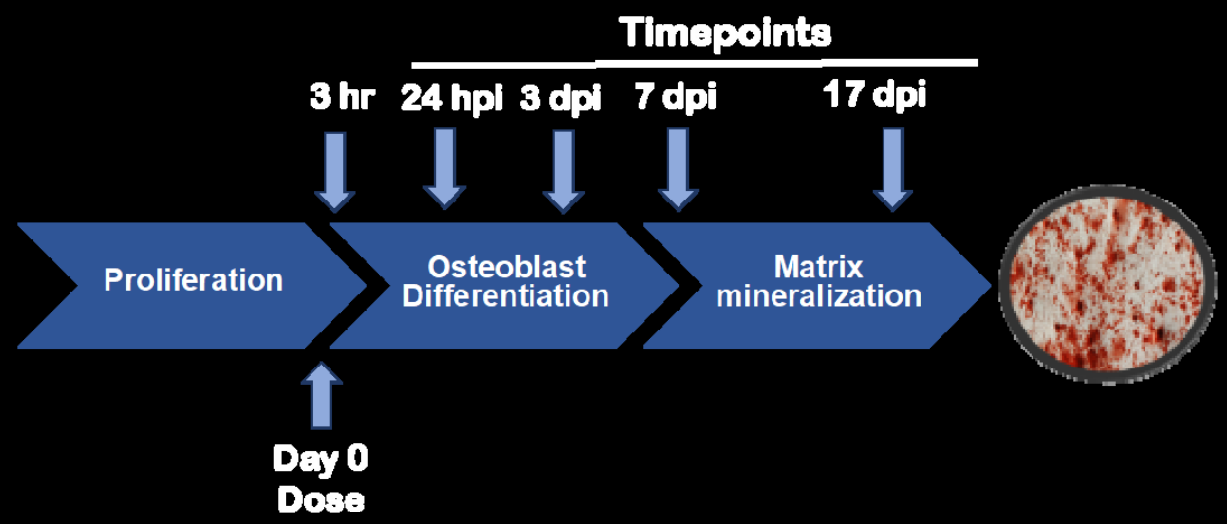

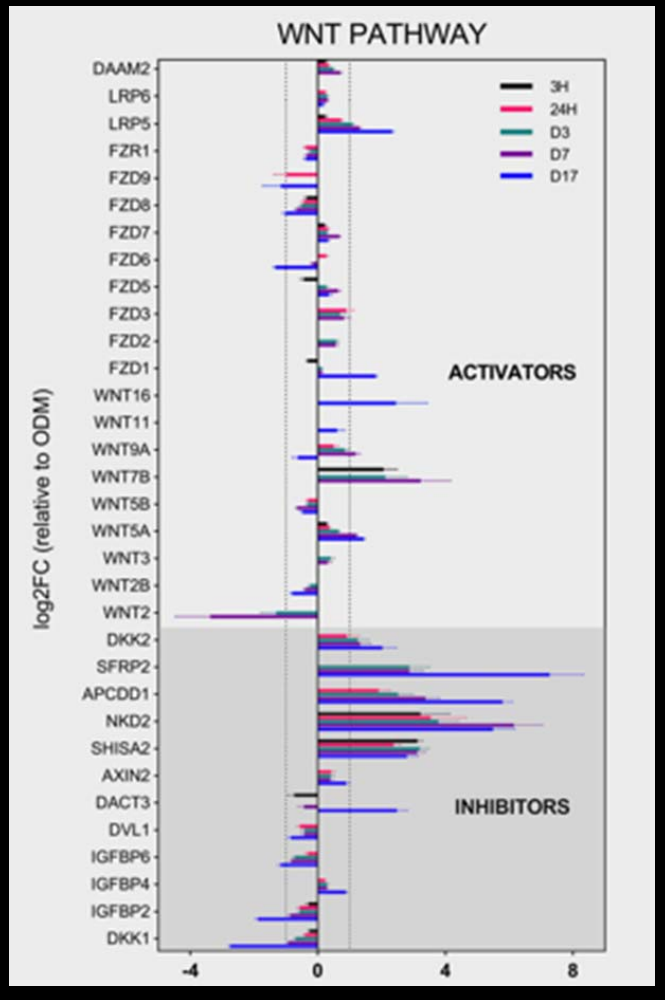

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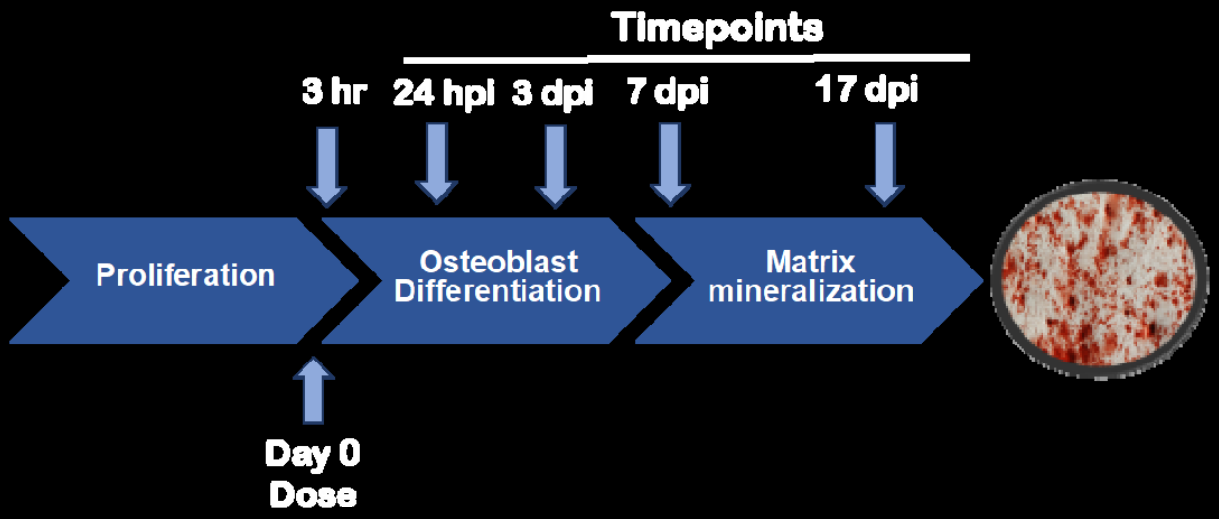
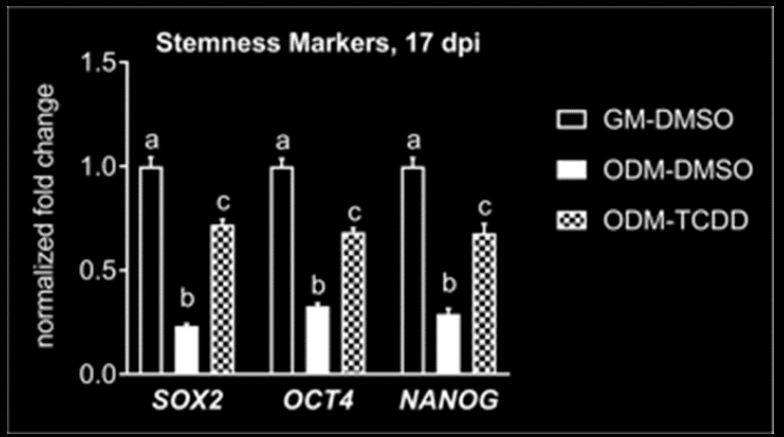
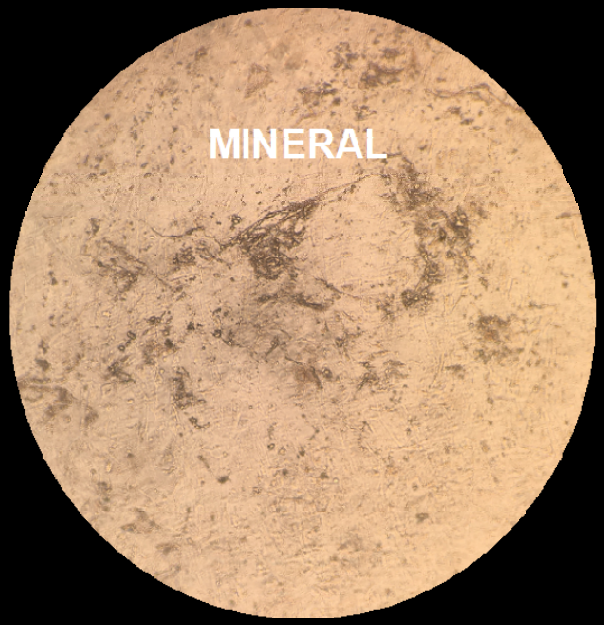
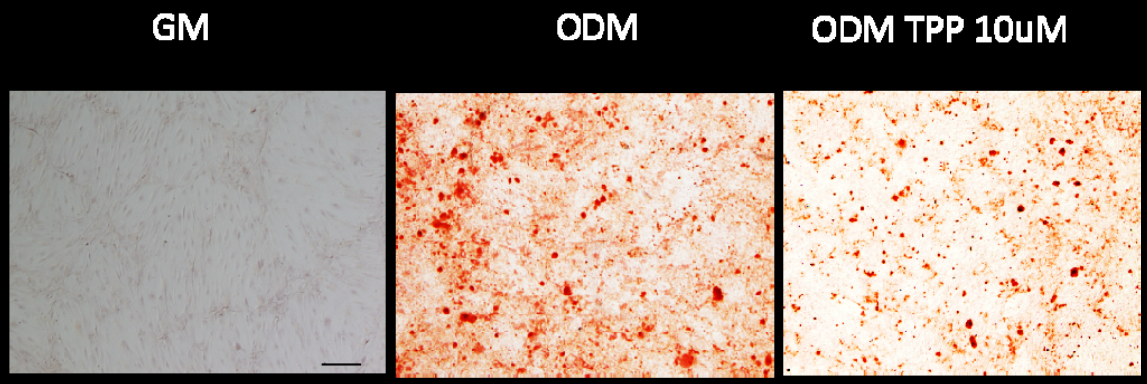




TPP: Attenuates Osteogenic Differentiation

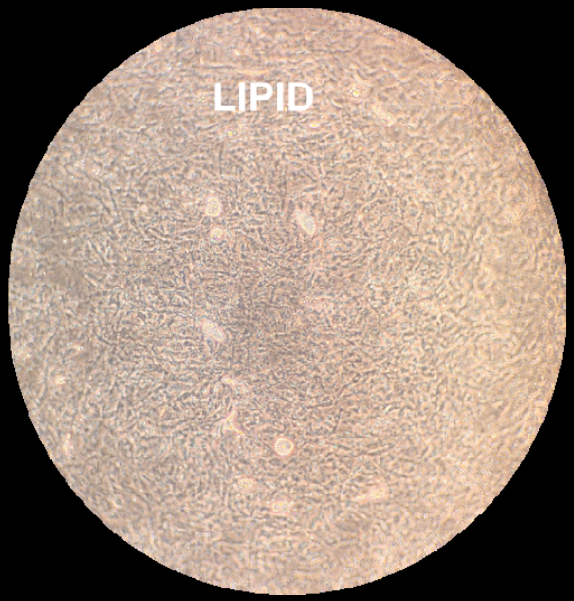
hMSC

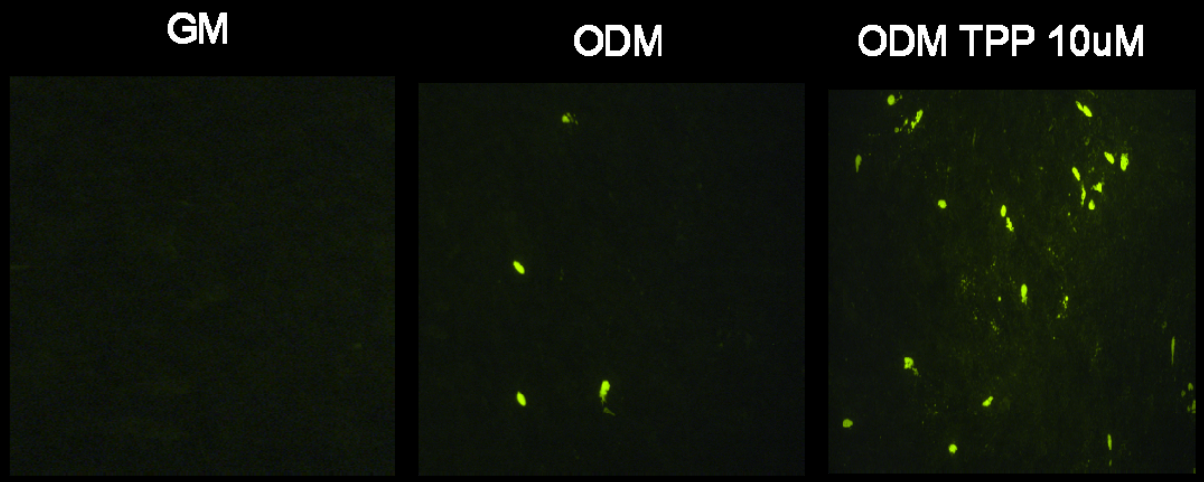




hMSC

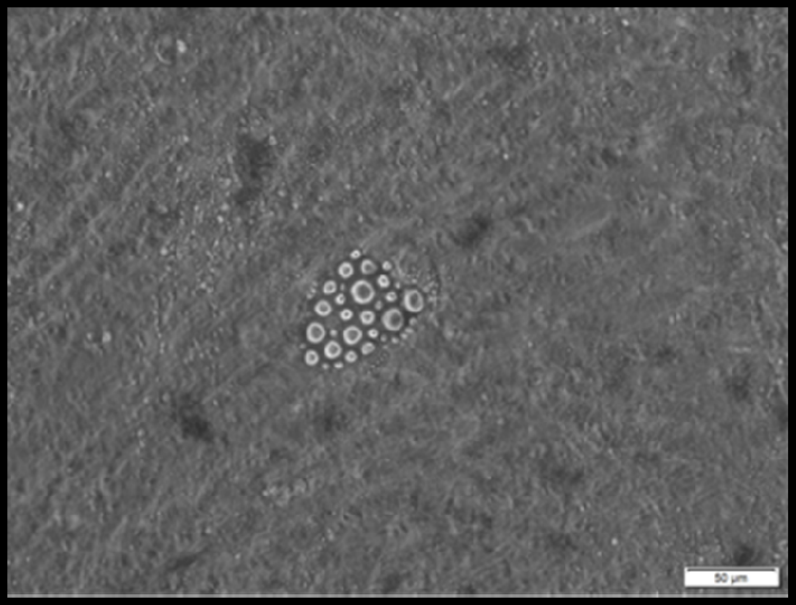
TPP: Induces Formation of Adipocytes Under Osteogenic Conditions



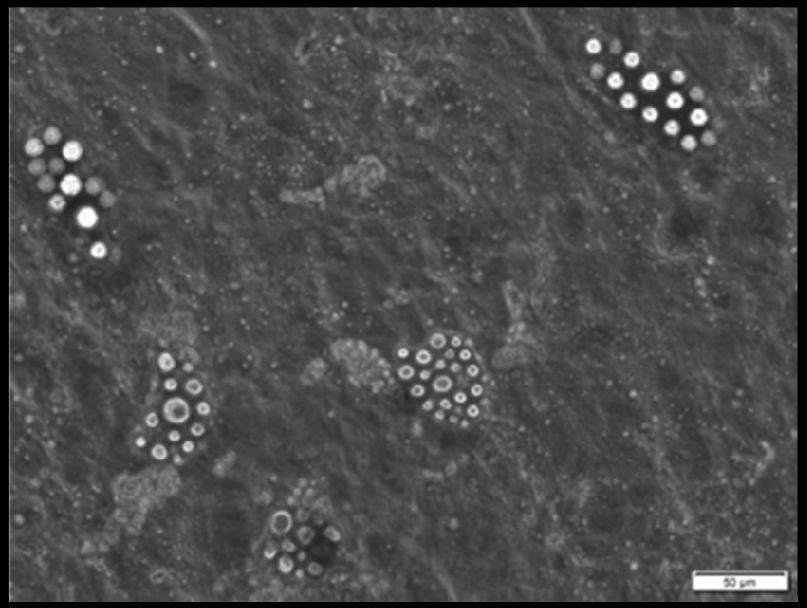


hMSC

TPP + Insulin: Enhances Adipocyte Formation Under Osteogenic Conditions

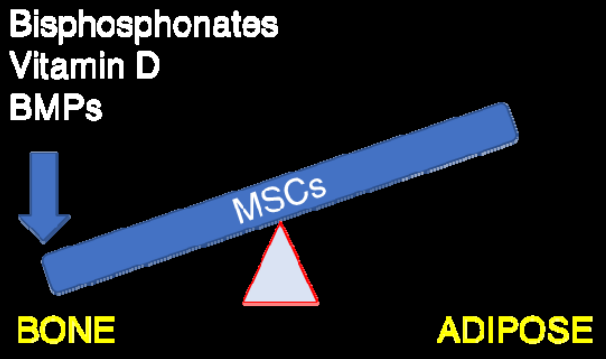


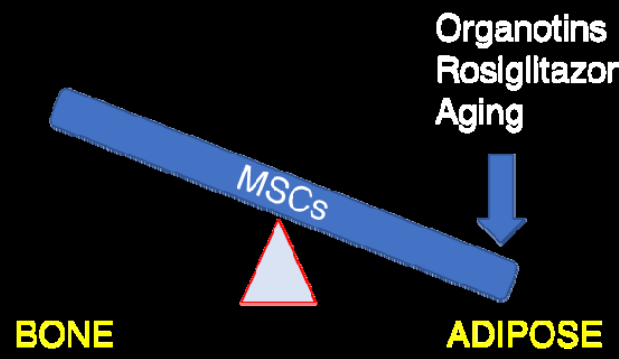
ODM control
+ insulin



TPP 10 µM
+ insulin

MSC Multipotency – a Cellular Seesaw in Response to Endogenous/Exogenous Agents





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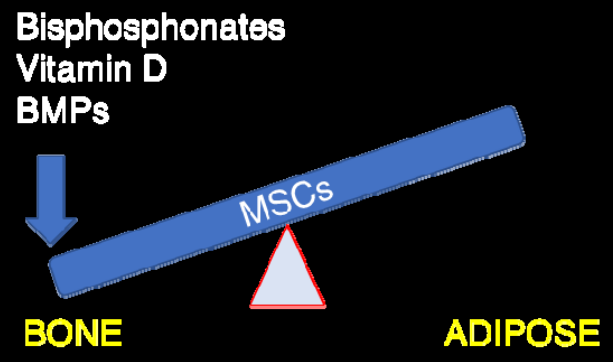

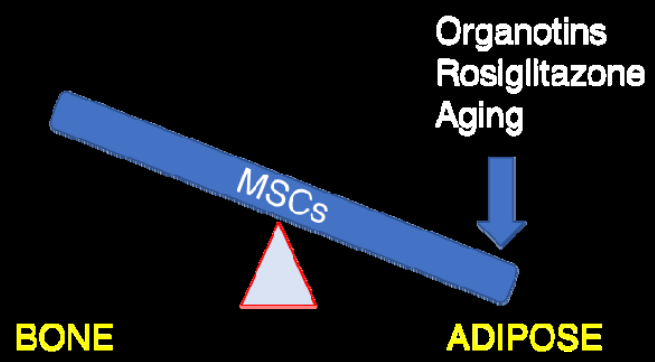

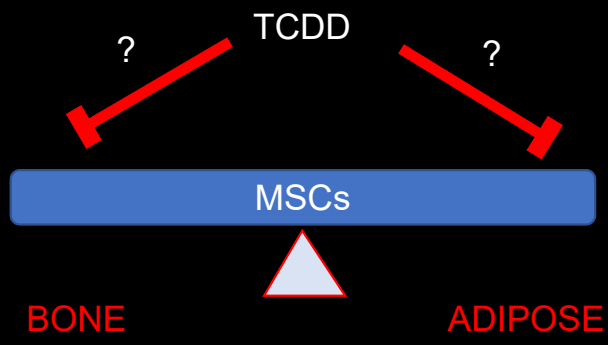

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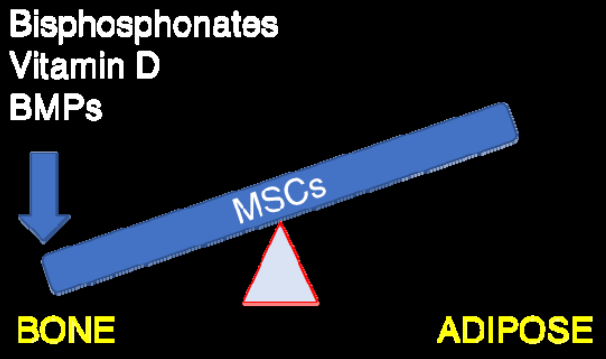

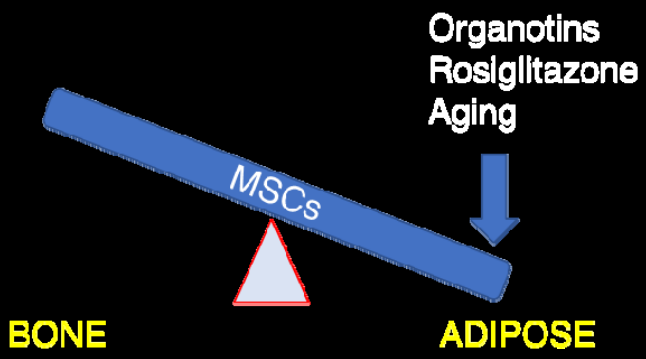

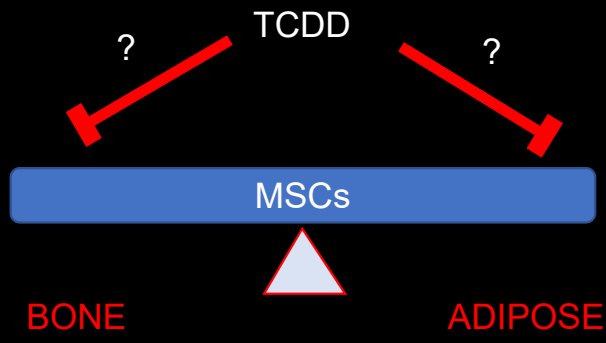

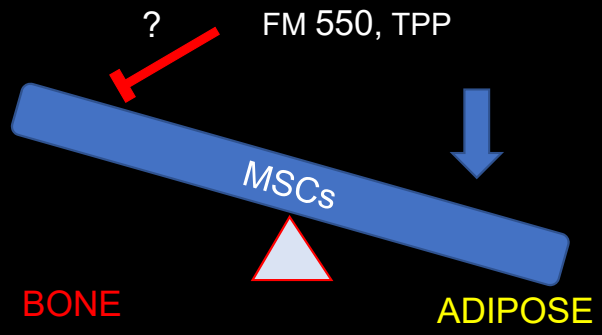

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A few salient points…

- Developmental exposures to FRs can significantly disrupt cartilage, bone morphogenesis in vivo.
- Phenotypes in SAF serve as excellent models for linkage of phenotypic and molecular changes that may mimic select skeletal dysplasia / diseases.
- FRs likely impact cell lineage progression and/or differentiation of MSCs with varying mechanisms.
- In process of identifying FR targets including NR's, and other proteins in collaboration with CPI.

Acknowledgements

Stapleton Lab

- Christopher Kassotis, PhD
- Erin Kollitz, PhD
- Kate Hoffman, PhD
- Lee Ferguson, PhD
- Allison Phillips, PhD
- Stephanie Hammel, PhD
- Kirsten Overdahl
- Samantha Hall
- Emina Hodzic
- Matt Ruis
- Sharon Zhang

Kullman Lab

- AtLee Watson, PhD
- Stacy Schkoda
- Chris Kassotis, PhD
- Megan Knuth
- Melissa Gronske
- Morgan Ritter

Sean Ekins, PhD. Collaborations Pharmaceutical Inc.

National Institute of Environmental Health Sciences

Grants:

NIH: P42 ES010356
NIH: T32 ES007046
NIH: P30 ES025128