

In search for obesogens

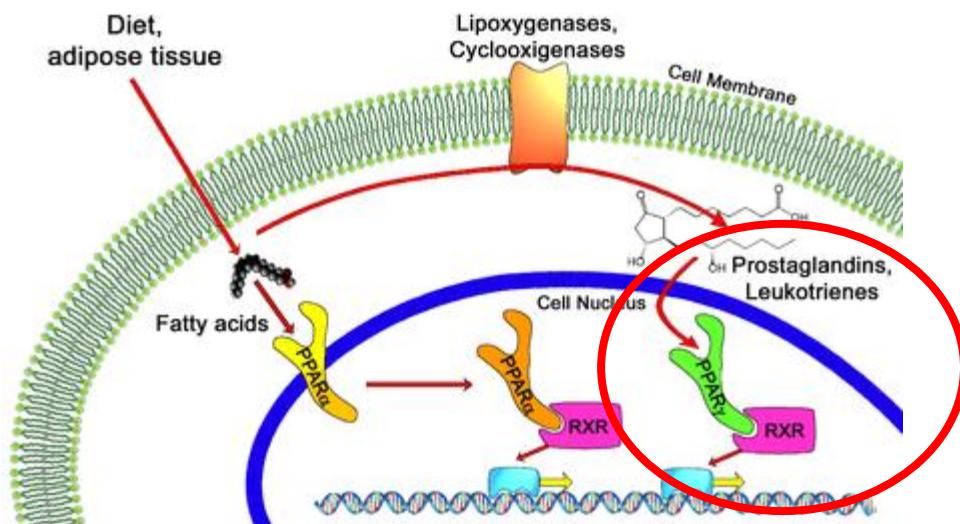
Ewa Szalowska

BDS Amsterdam 2012

Obesogens-an environmental link to obesity

Obesogens (B. Blumberg 2006)- a subset of endocrine disrupting chemicals (EDCs) that activate PPAR γ

PPAR γ is one of the nuclear receptors' ligand-activated transcription factor that mediates adipogenesis and maintenance of mature adipocytes



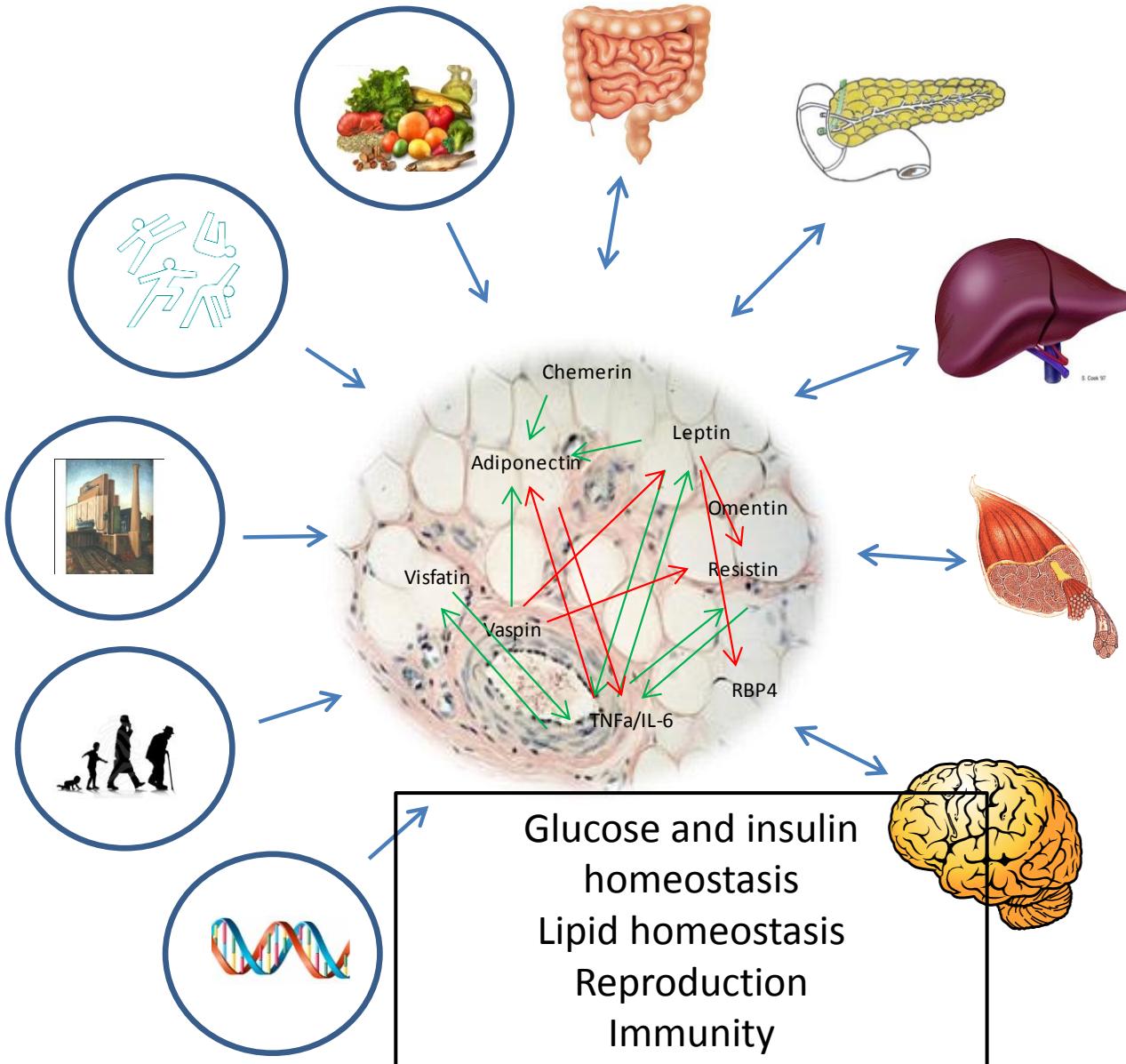
PPAR γ gene targets:

PPAR γ 2, FABP4, LPL, CD36, adiponectin, Klf15



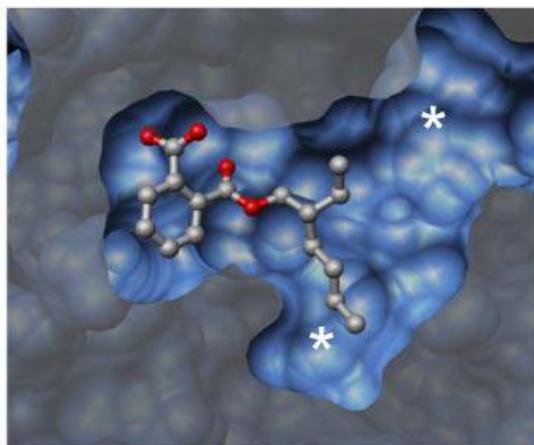
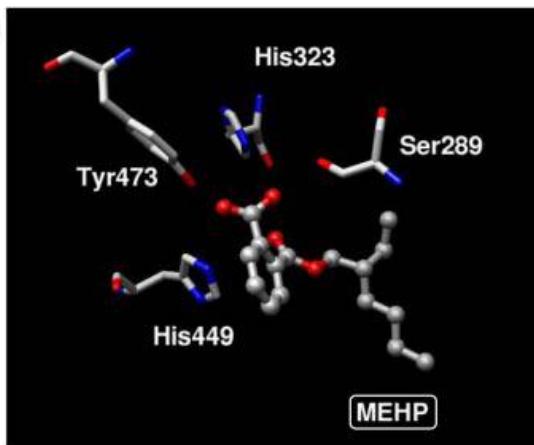
Adipocytes differentiation & maintenance

Adipose tissue energy homeostasis

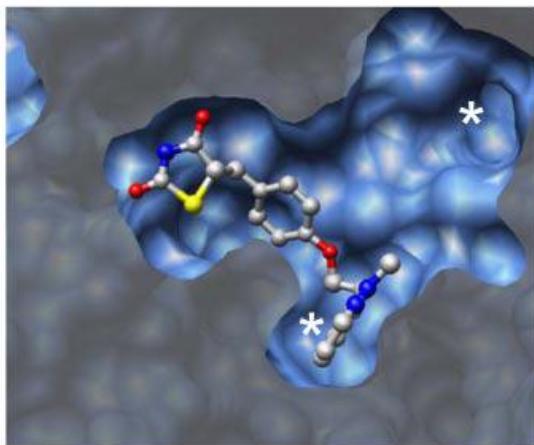
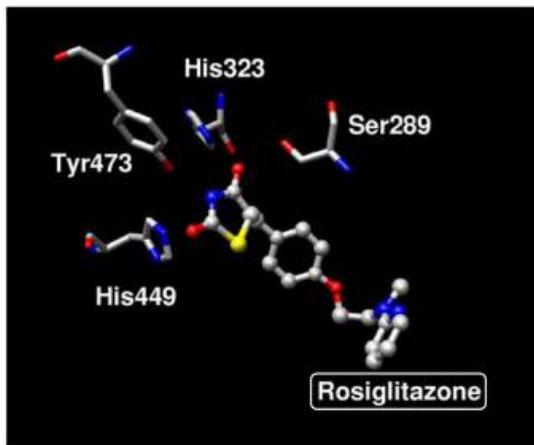


MEHP and rosiglitazone bind similarly to the PPAR ligand-binding domain

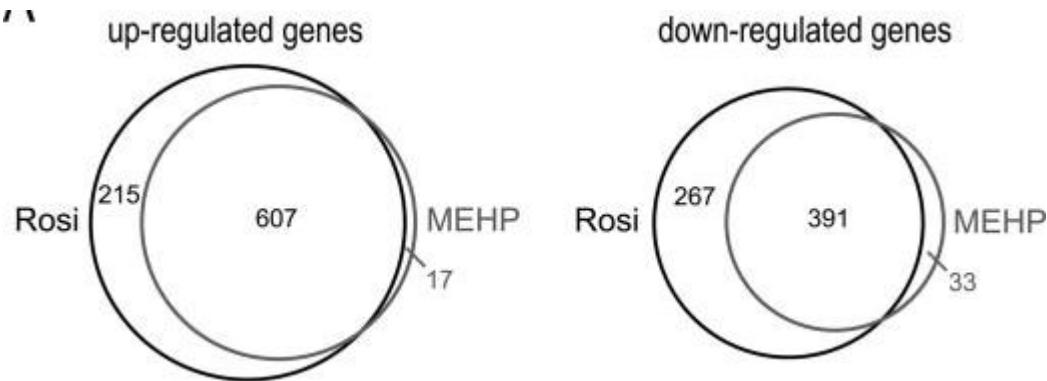
A



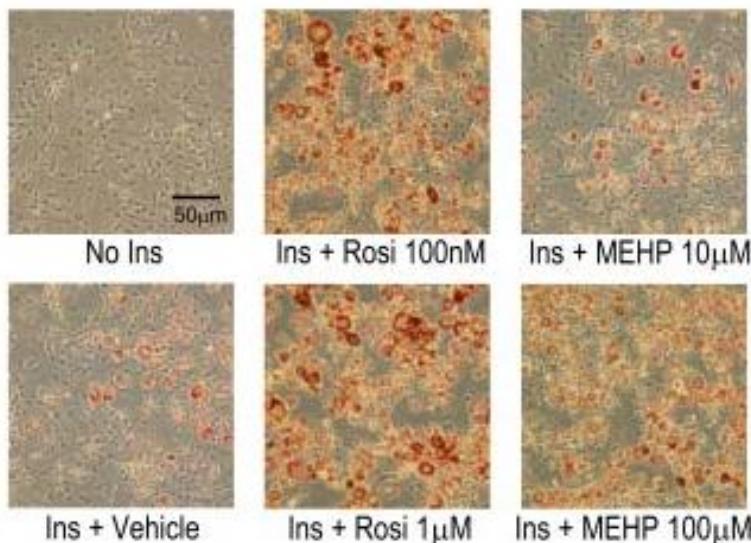
B



MEHP regulates only a subset of genes compared with rosiglitazone



MEHP induces adipogenesis through PPAR γ

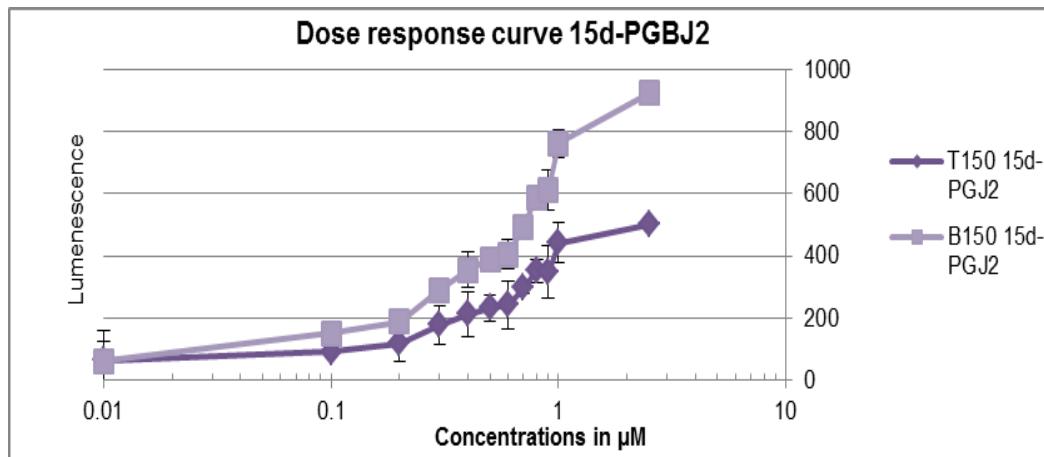
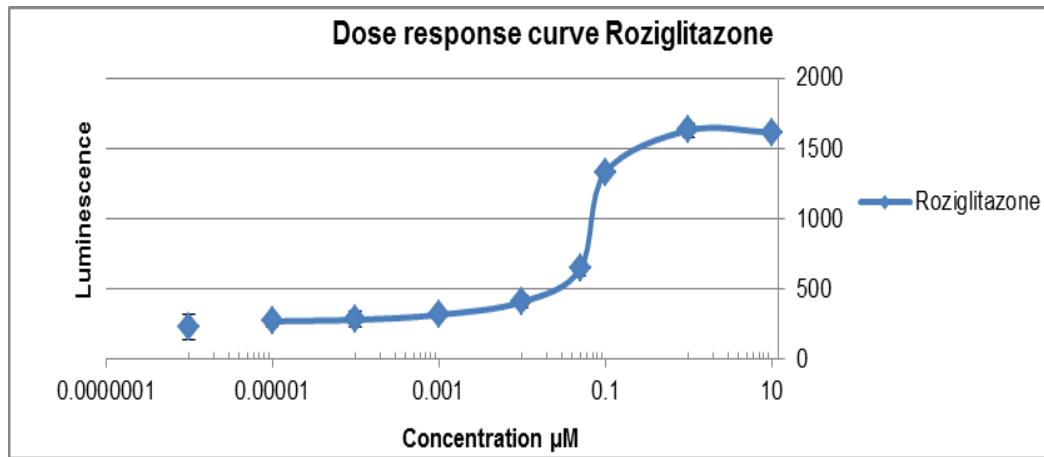


Feige JN, et al. The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor g modulator that promotes adipogenesis. JBC 282:26:19152-19166 (2007)

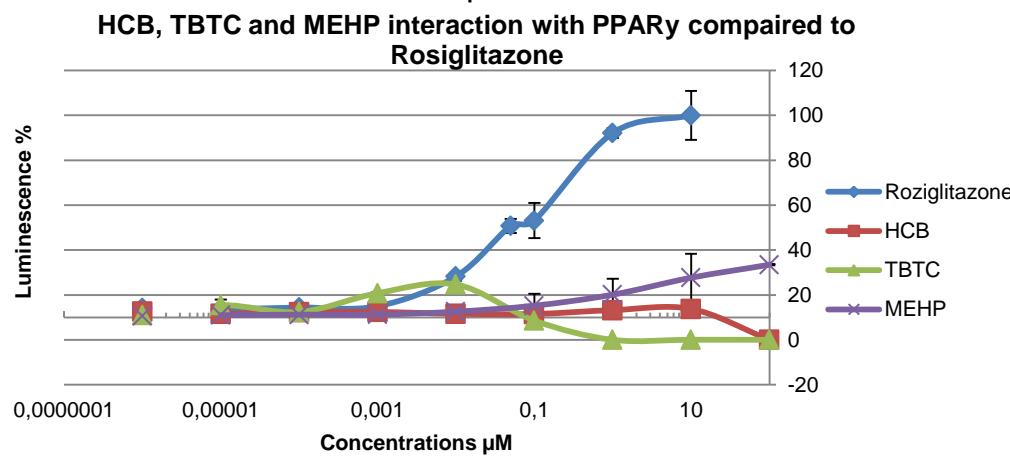
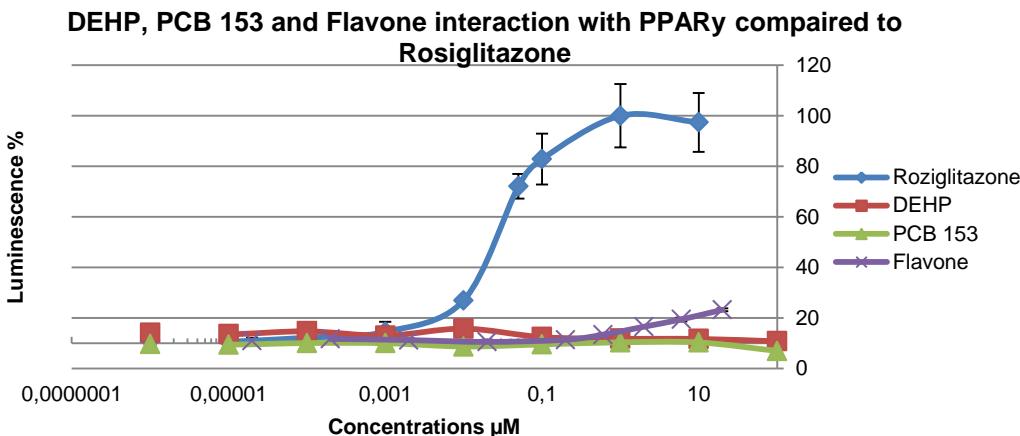
Obesogens selection

Compound	Concentrations µM	Product
Rosiglitazone (positive control)	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Anti Diabetes
15d-PGJ2 prostaglandin (positive control)	$1 \cdot 10^6 > 1 \cdot 10^5$ µM	Natural agonist of PPAR γ
Flavone (positive control)	$2 \cdot 10^5 > 2 \cdot 10^1$ µM	Natural compound
Ethinyl-Estradiol (negative control)	$1 \cdot 10^5 > 3, 1$ µM	Hormone
DDT (dichlorodiphenyltrichloroethane)	$4 \cdot 10^4 > 2$ µM	Organochlorine, insecticide
DDE (Dichlorodiphenyldichloroethylene)	$1 \cdot 10^4 > 2$ µM	Product of DDT
β HCH (β -hexachlorocyclohexane)	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Pesticide product, organochloride
DES (Diethylstilbestrol)	$4 \cdot 10^4 > 2$ µM	Synthetic estrogen
Bisphenol A/B/C	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Plasticizers
PCB 153 2,2',4,4',5,5'-Hexachlorobiphenyl	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Environmental contaminant
DEHP (Bis(2-ethylhexyl)phthalate)	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Plasticizer
MEHP (mono-2-ethylhexyl phthalate)	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Plasticizer
TPTC tri-n-propyltin chloride	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Biocides, organotin
TBTc Tributyltin chloride	$0.1 > 0.000000001\%$	Biocides, organotin
HCB β -hexachlorocyclohexane	$1 \cdot 10^5 > 1 \cdot 10^{-3}$ µM	Fungicide, organochloride, insecticide

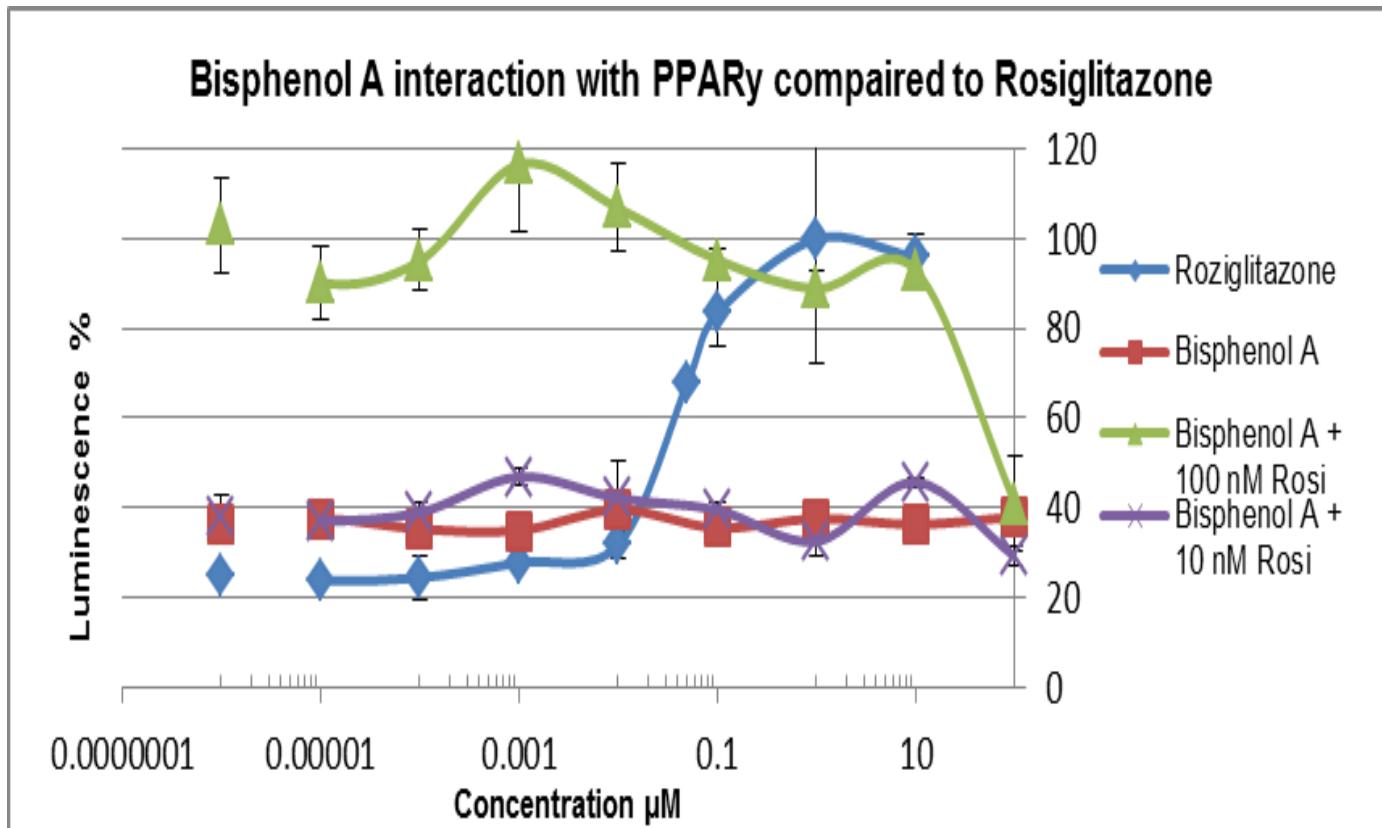
PPAR γ 2 CALUX



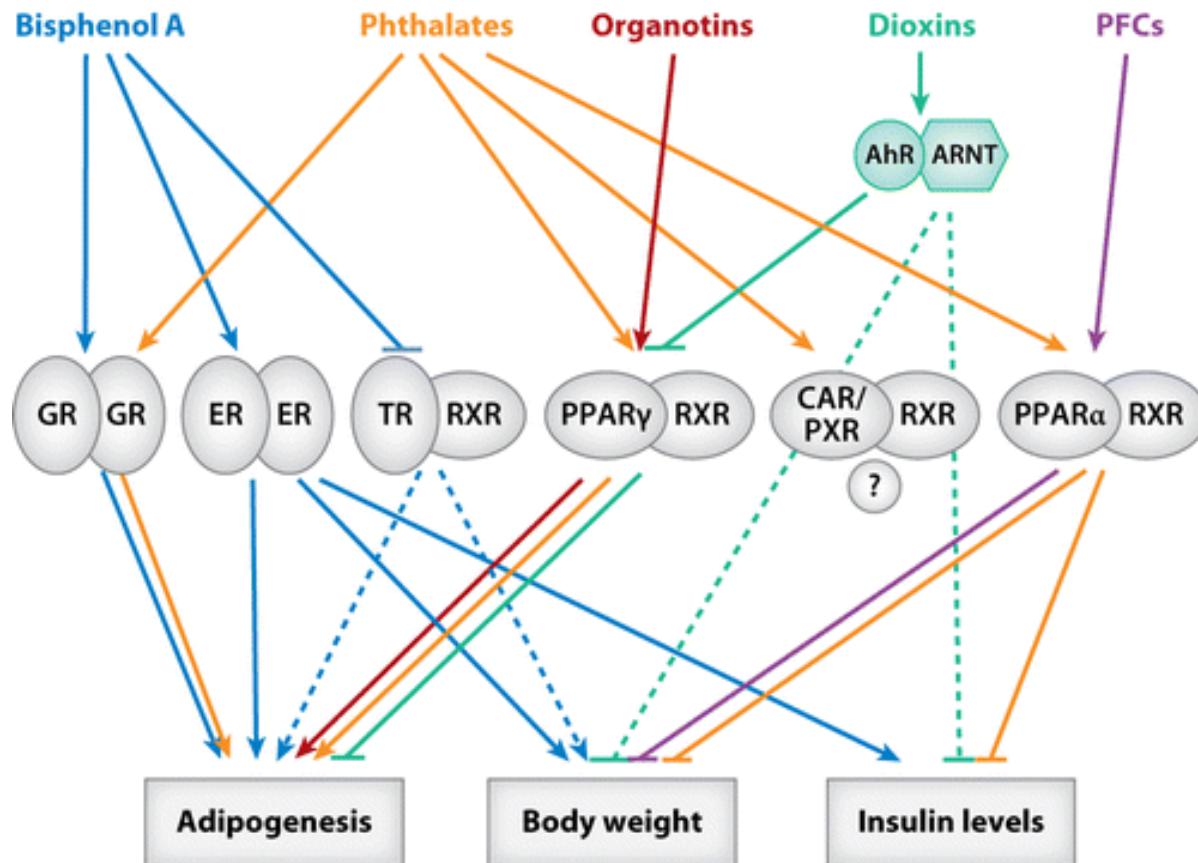
DEHP, PCB, Flavone-PPAR γ CALUX



Bisphenol A -PPAR γ CALUX



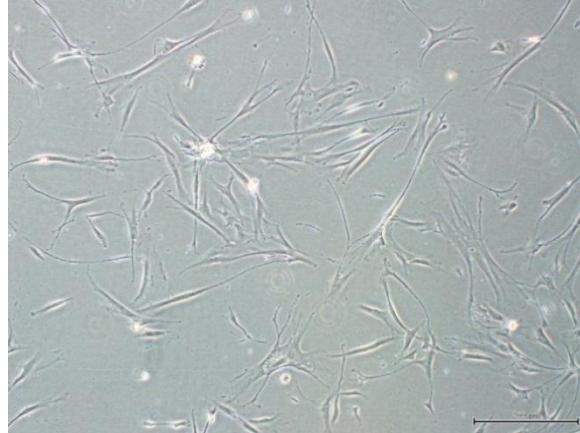
Obesogens beyond PPAR γ



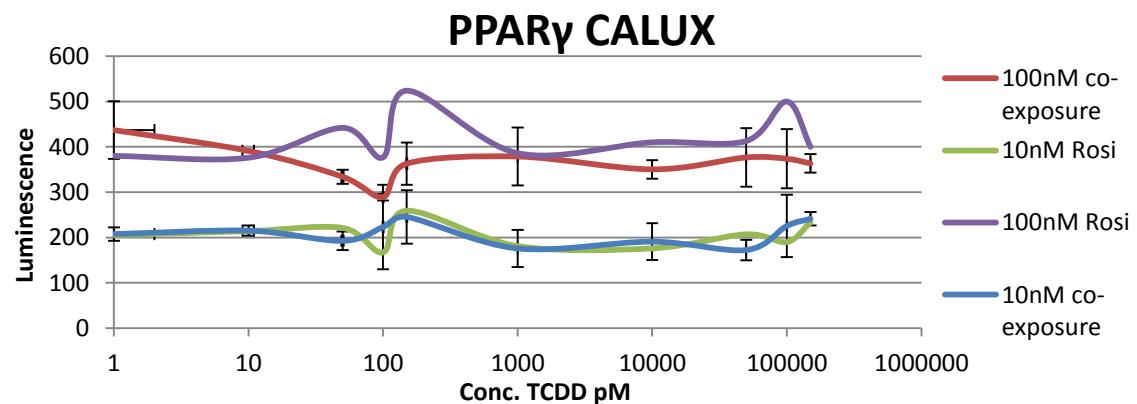
Human Simpson-Golabi-Bheme-Syndrome (SGBS) cells

Advantages:

- Human
- More efficient to differentiate
- Unlimited source

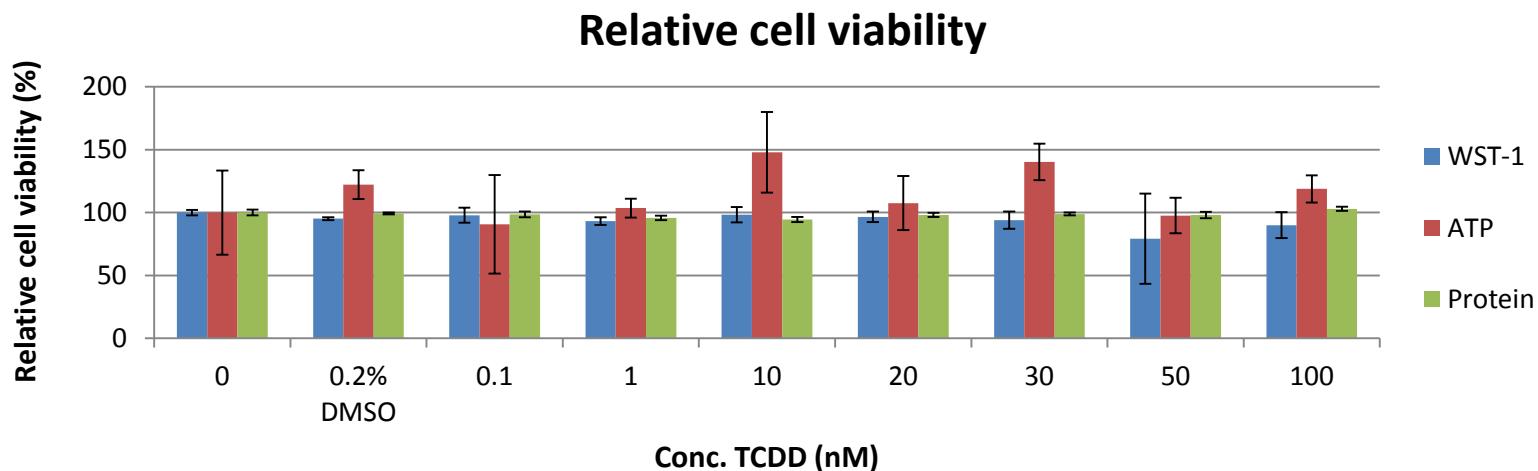
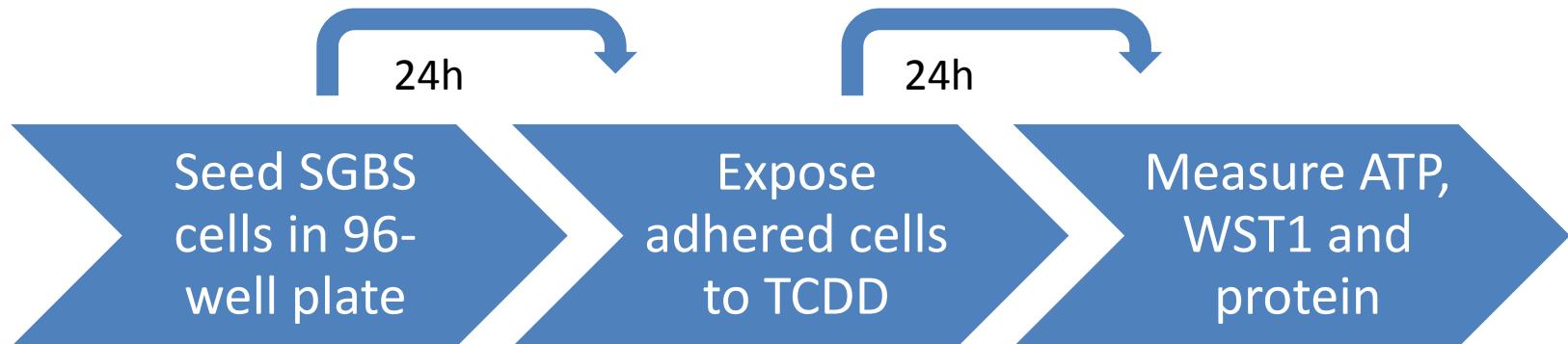


No direct effect of TCDD on PPAR γ

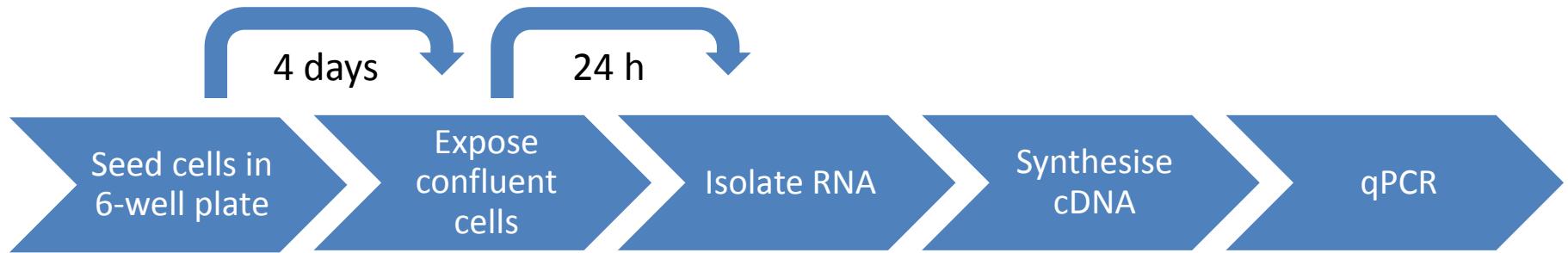


No antagonistic effect

Cytotoxicity



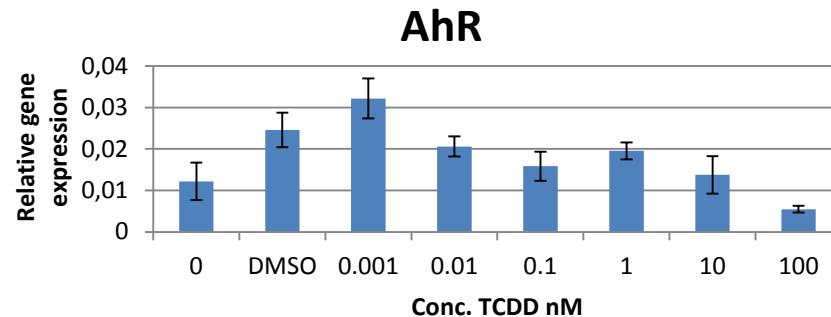
Dose dependent acute effect of TCDD on gene expression in undifferentiated SGBS cells



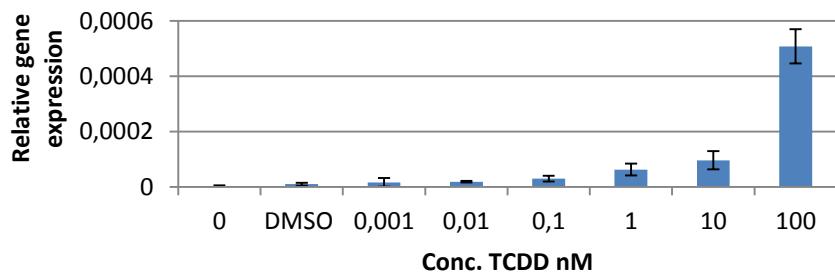
TCDD target genes: AhR, CYP1A1, CYP1B1

Inflammatory genes: MCP1, IKK β , Serpin B2, IL-6, IL-1 β , TNF α

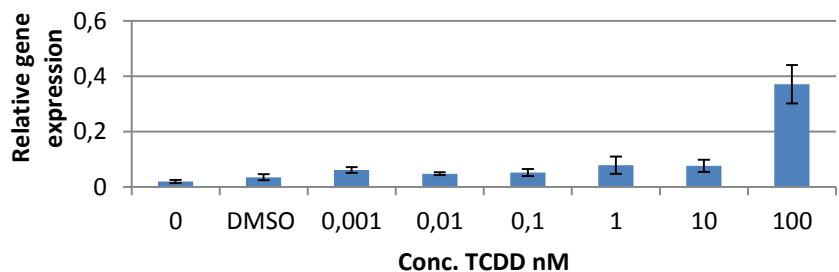
TCDD target gene expression



CYP1A1

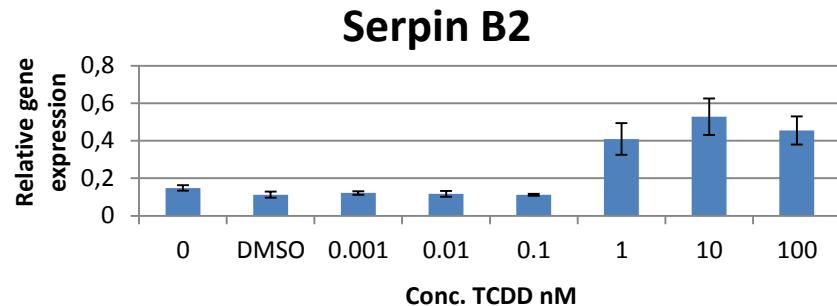
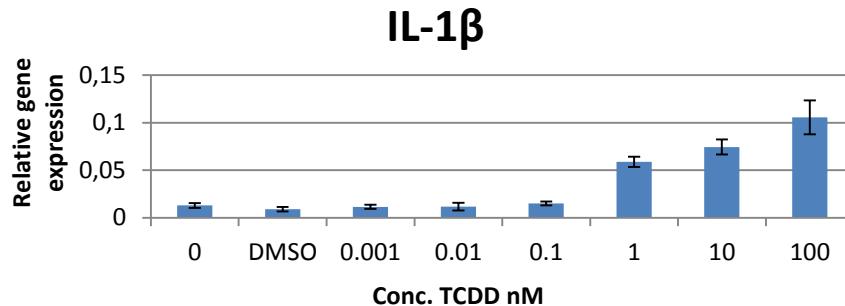
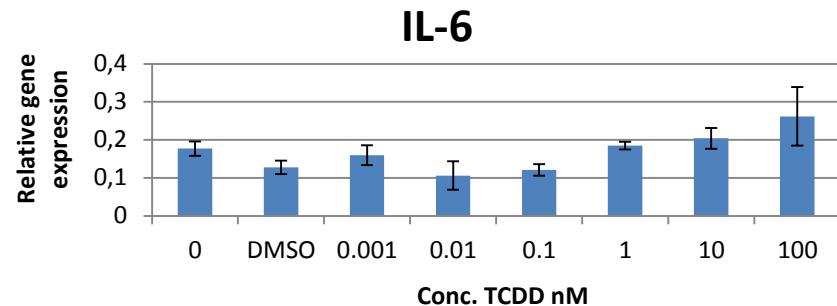
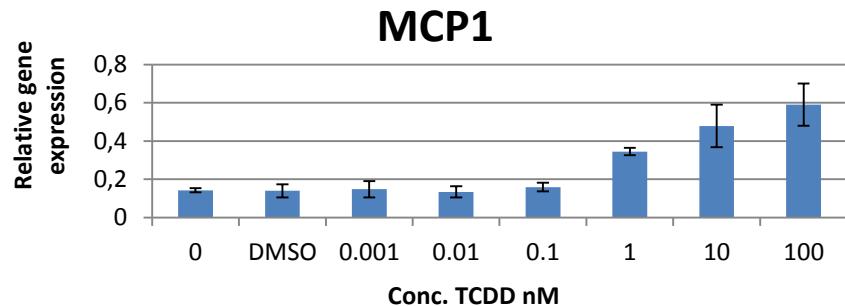


CYP1B1



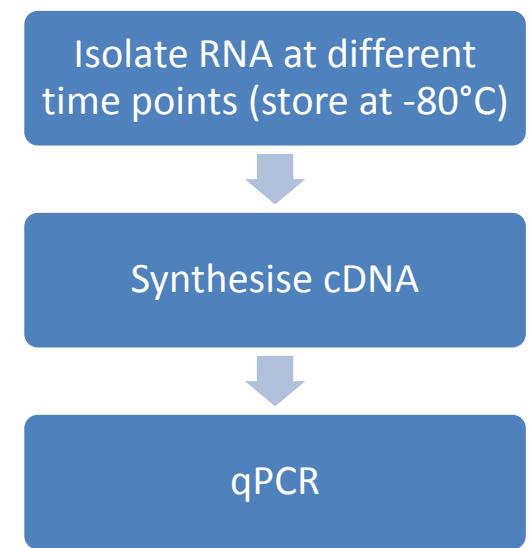
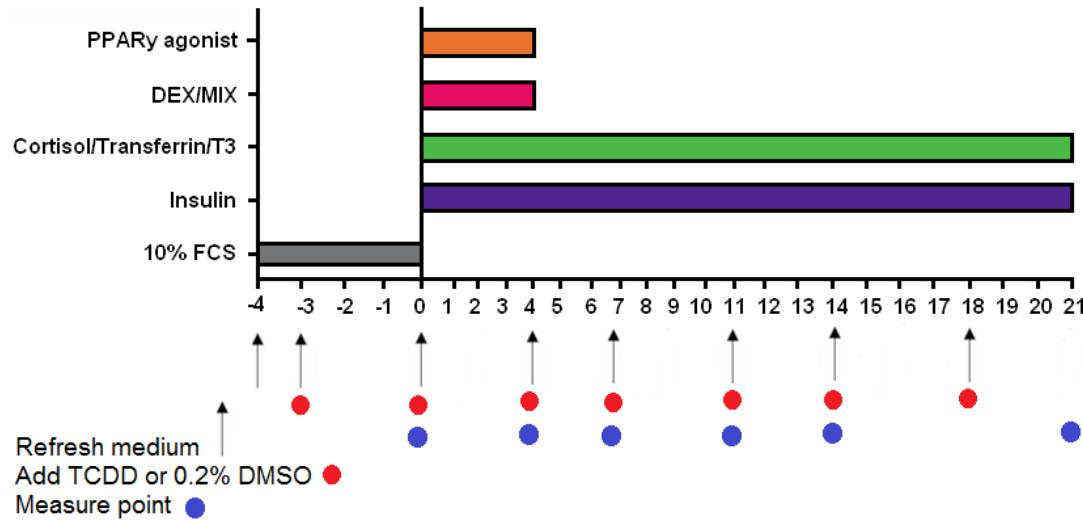
100nM TCDD down-regulates AhR expression and up-regulates CYP1A1 and CYP1B1 expression

Inflammatory gene expression



1nM TCDD and higher induces dose-dependent up-regulation of inflammatory marker gene expression

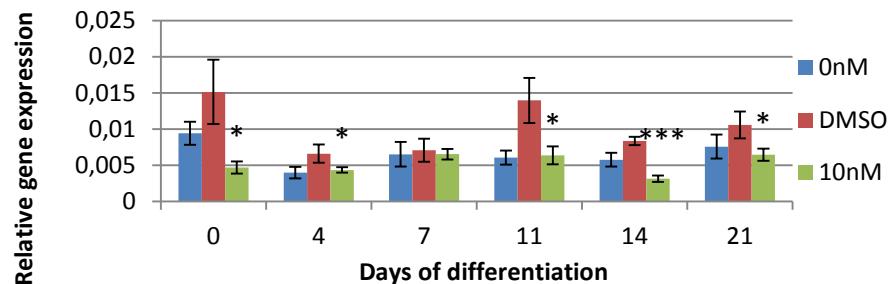
Chronic effect of 10nM TCDD on gene expression during differentiation



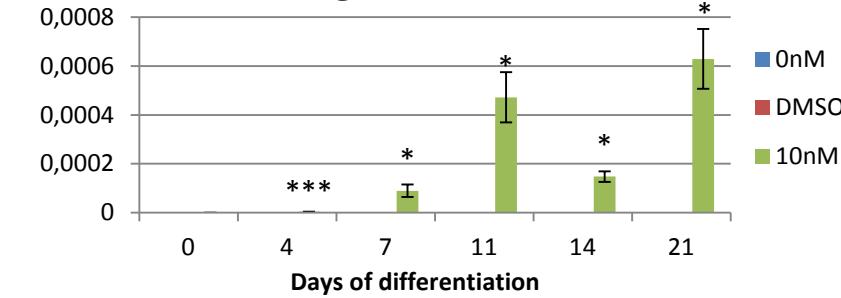
TCDD target genes
Inflammatory marker genes
Adipogenic (metabolic) genes: PPAR γ , Adiponectin, Leptin, LPL, FABP4

TCDD target gene expression

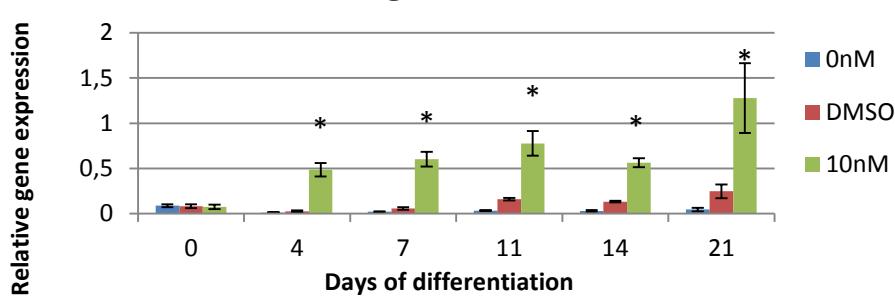
AhR



CYP1A1

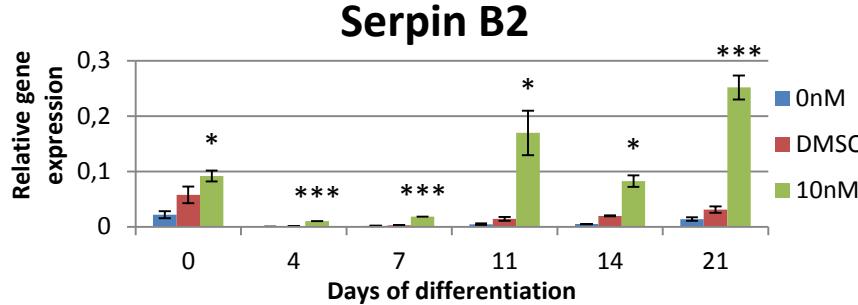
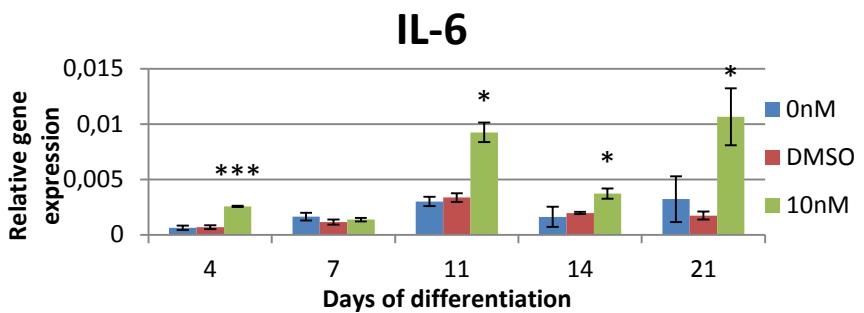
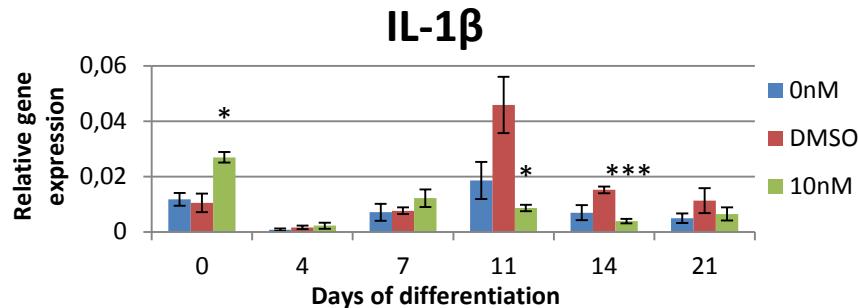
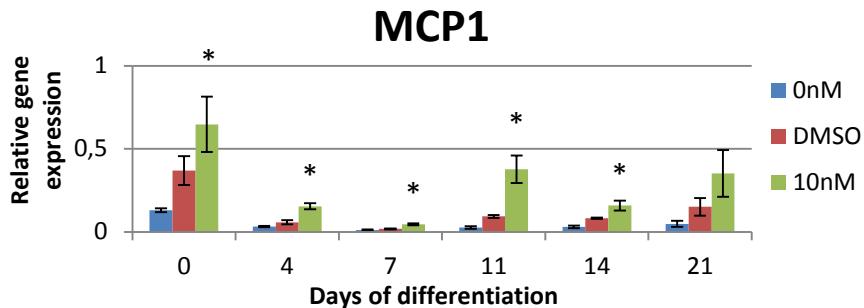


CYP1B1



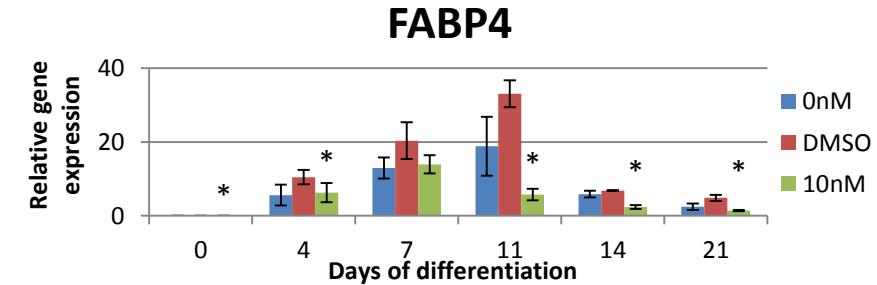
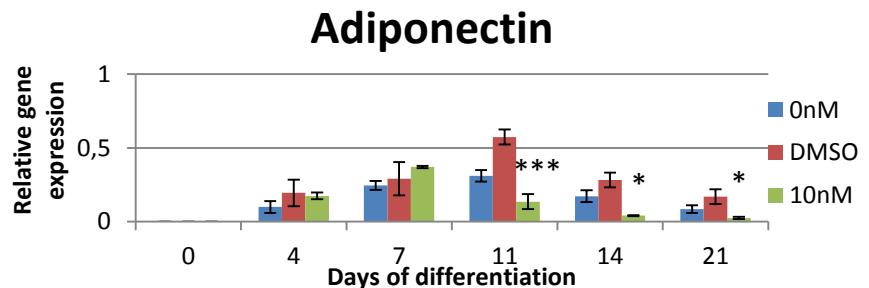
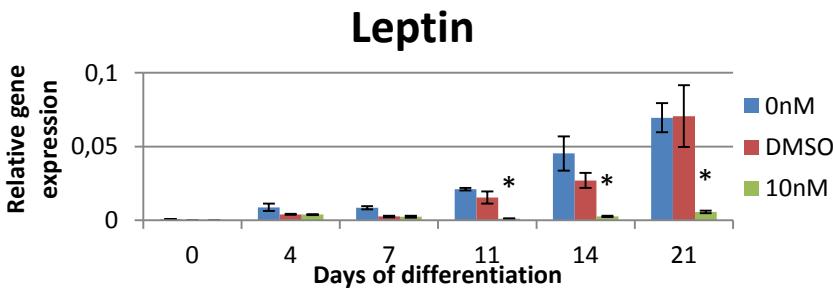
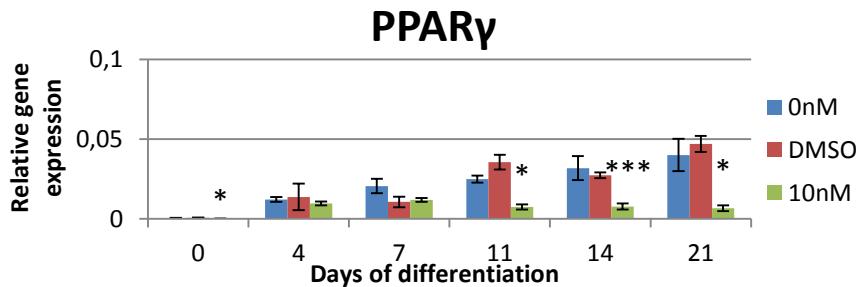
TCDD down-regulates AhR expression and up-regulates CYP1A1 and CYP1B1 expression

Inflammatory gene expression



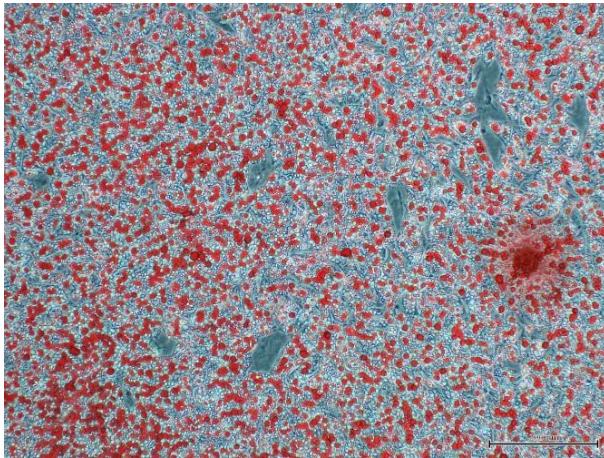
TCDD up-regulates most of the inflammatory marker gene expression

Adipogenic (metabolic) gene expression

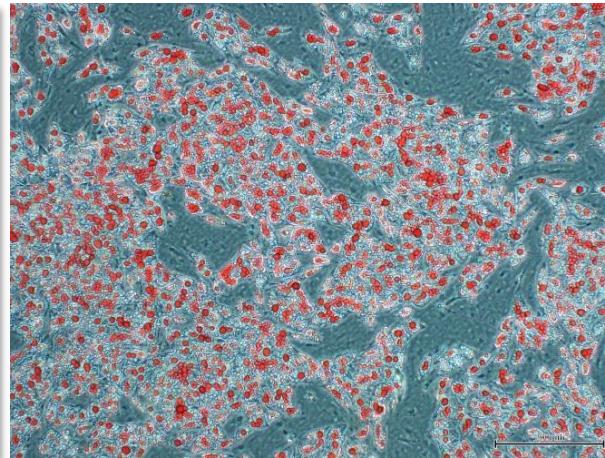


Oil Red O staining

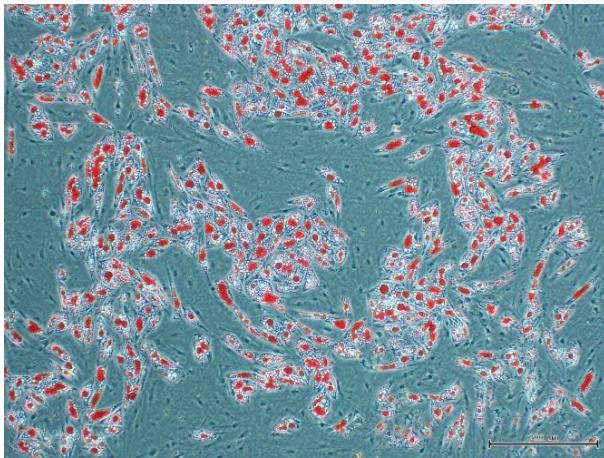
0nM



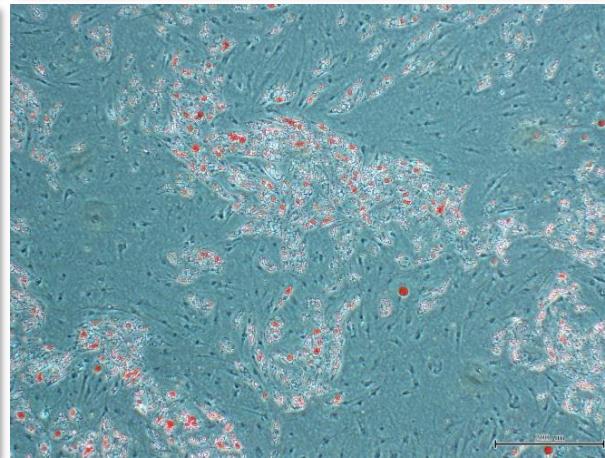
10nM



50nM

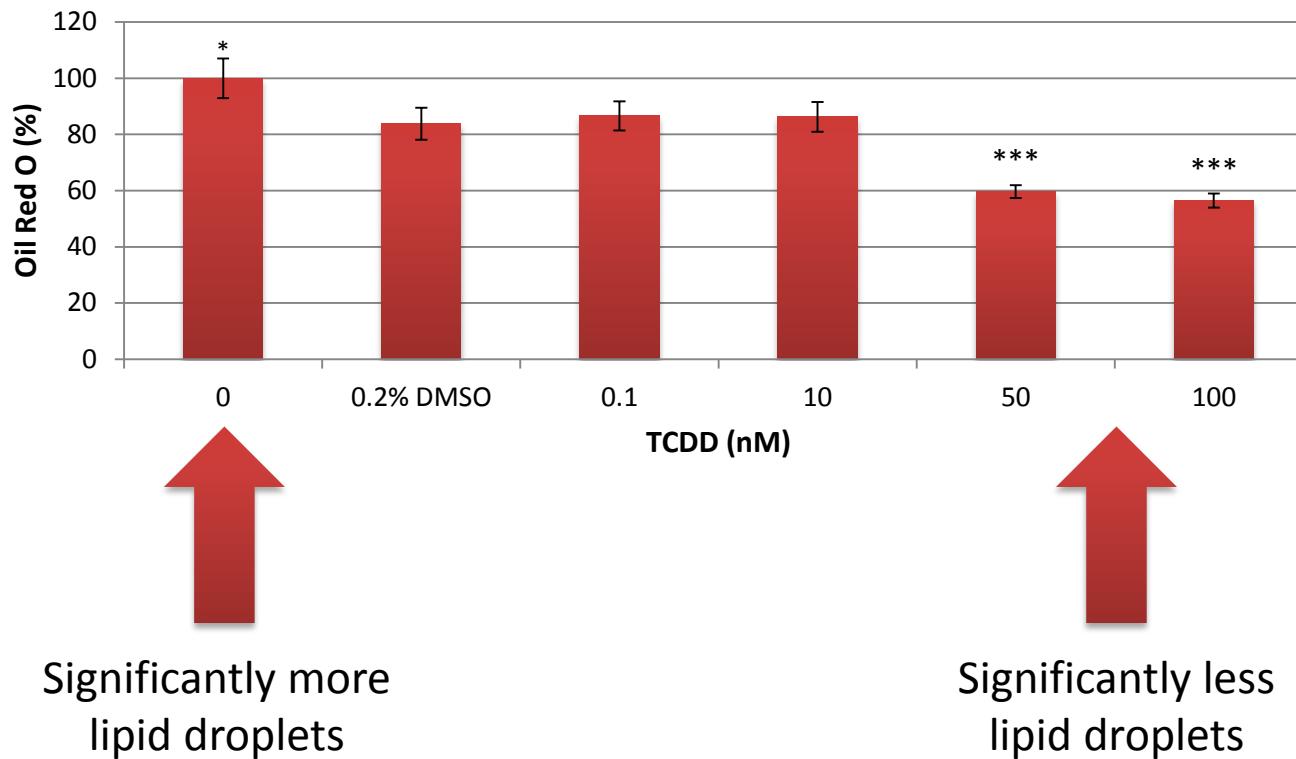


100nM



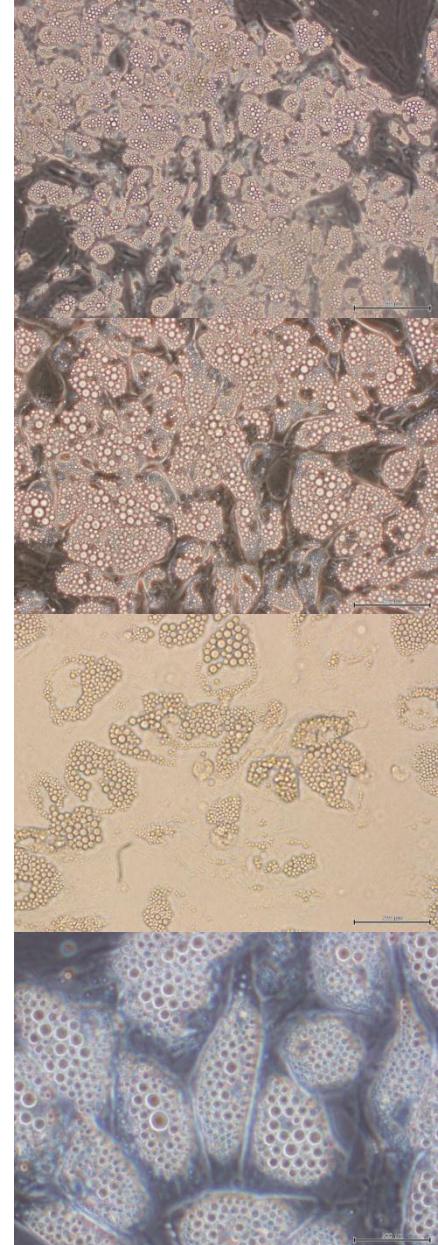
Dose dependent decrease of TCDD on the amount of lipid droplets

Oil Red O quantification



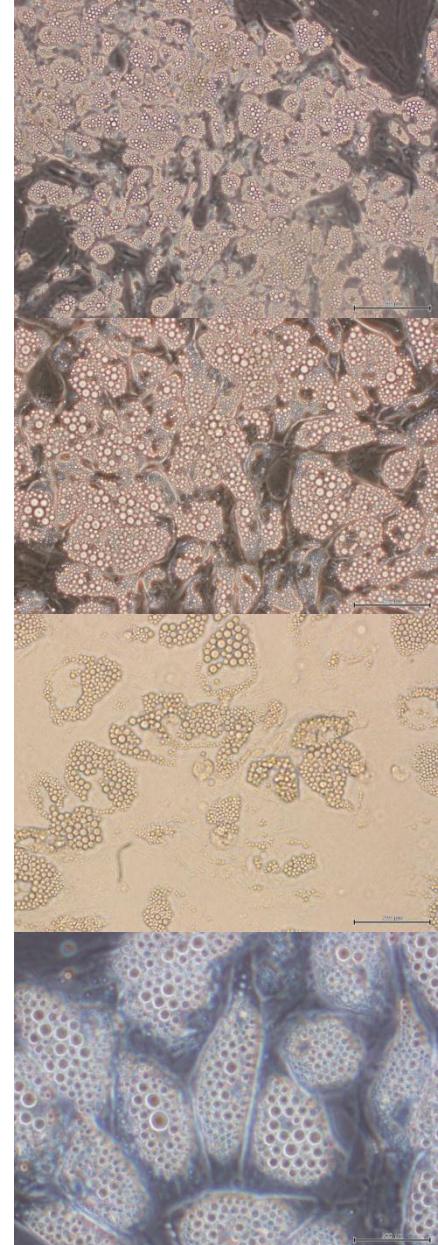
Conclusion

- TCDD despite clear toxicity significantly alters gene expression
- These changes could lead to functionally altered adipocytes that are predisposed to metabolic disorders



Conclusion

- PPAR γ CALUX is successful to identify subset of obesogens
- SGBS cell line can be used to identify larger number of obesogens/EDCs



Thanks to :

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Prof. Martin Wabitsch (University of Ulm)

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