# Ah Receptor-Mediated Disruption of the Cardiac Embryogenesis Program

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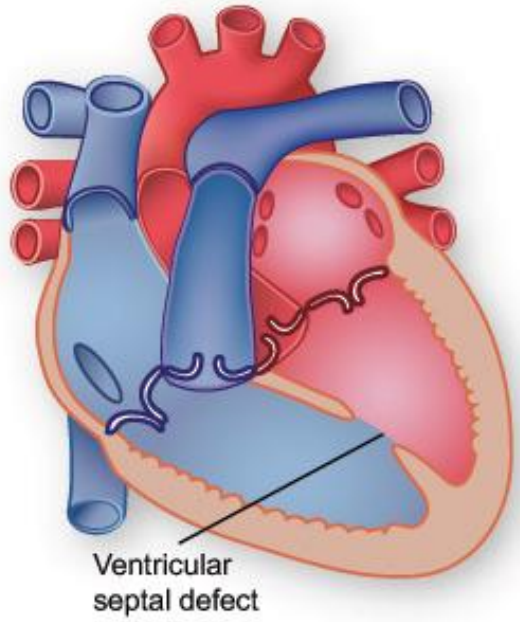
# Congenital Heart Disease

## Most prevalent form of human birth defect

- **30% of prenatal loss**
- **0.4%–5% of live births**
- **Most common: VSD**
- **High economical burden**
- $1.4 billion hospitalization cost per year (2013)

**Leading cause of adult cardiac insufficiency**




Source: texasheart.org

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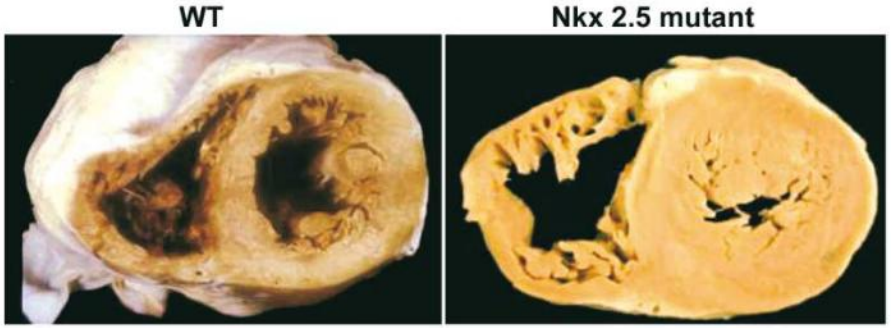
# Etiology of Congenital Heart Disease

**Genetic (explaining only ~15% of cases - CDC)**

- **Gene mutations**
  - » ***Cited2, Ets1, Foxh1, Gata4, Gata6, Hand1, Hand2, Hoxa1, Irx1, Nkx2-5, Nkx2-6, Pitx2*** **and** ***Tbx1***

**Environmental**

- **Metabolic**
- **Infectious**
- **Xenobiotics**
  - » ***Persistent organic pollutants (POP)***

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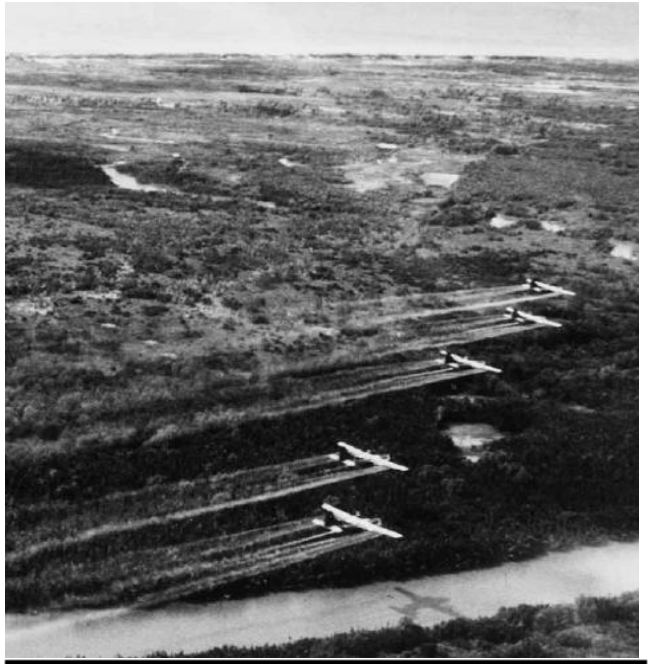
# At risk…

- Gestation in areas with high industrial, natural, or accidental release of POPs (e.g. dioxins)

- Important
  - Maternal lifelong dietary exposure to dioxins and dioxin-like compounds



**BIRTH DEFECTS RECOGNIZED BY THE VA AS CONNECTED TO AGENT ORANGE EXPOSURE**

**Spina Bifida:** children born to either male or female Vietnam veterans; Spina Bifida Occulta not included

**CHILDREN BORN TO FEMALE VIETNAM VETERANS**

**Achondroplasia:** produces a type of dwarfism

**Cleft Lip and Cleft Palate**

**Congenital Heart Disease**

+18 other diseases – VA Association

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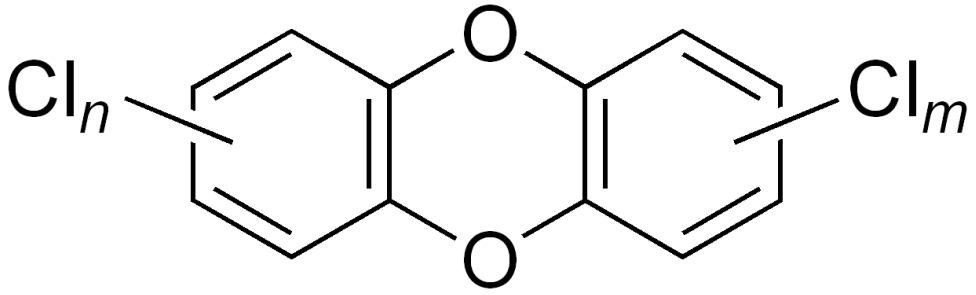
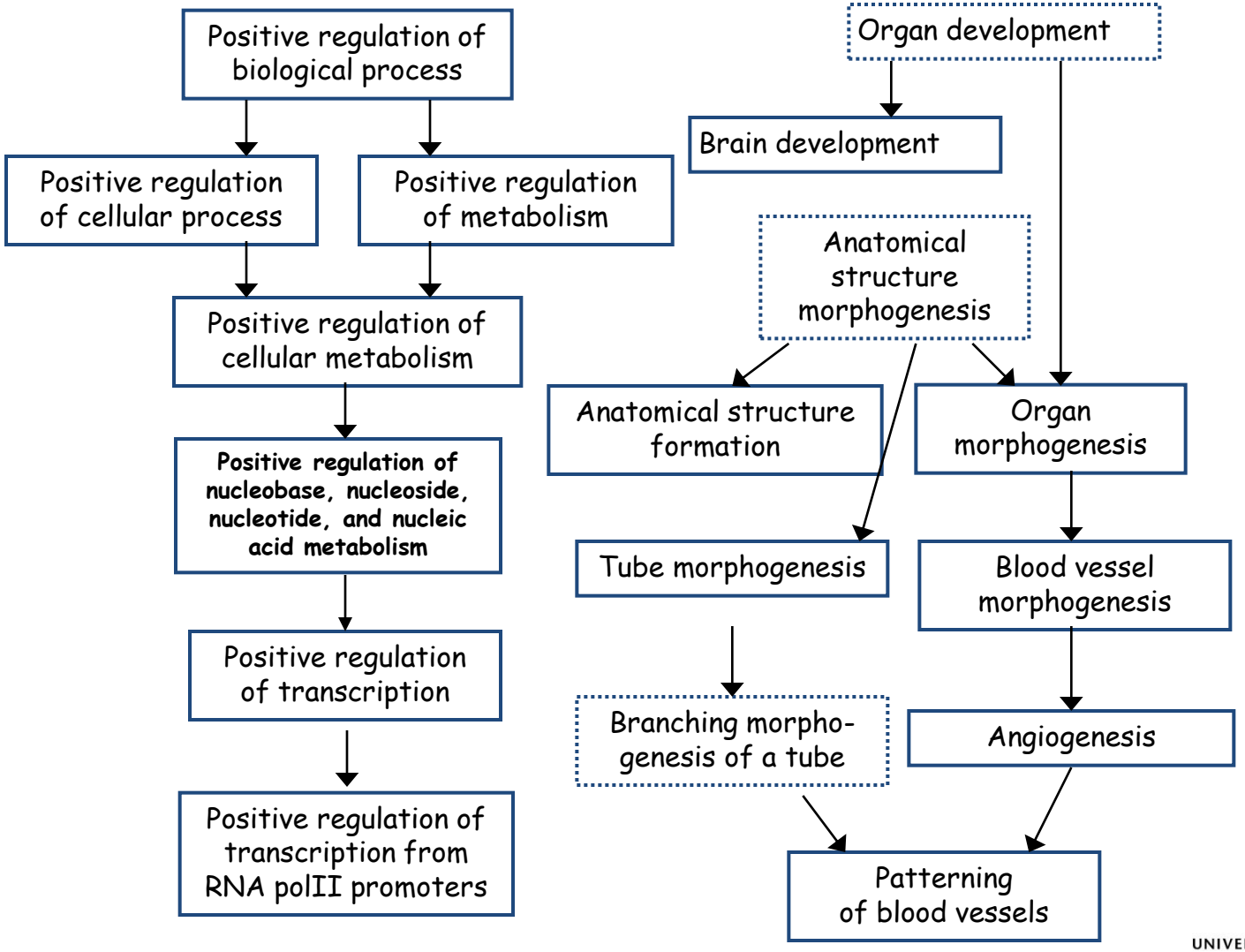
# Dioxins are potent agonists of the aryl hydrocarbon receptor (AHR)




- Dioxin (TCDD/TCDF) and Dioxin-like (PCBs)
  - At least 300-400 toxic compounds
- Dioxins share:
  - Structural relationship
  - Environmental persistence and bioaccumulation
  - Bind to and elicit AHR-dependent biochemical/toxic response

# Biological processes regulated by the naïve AHR

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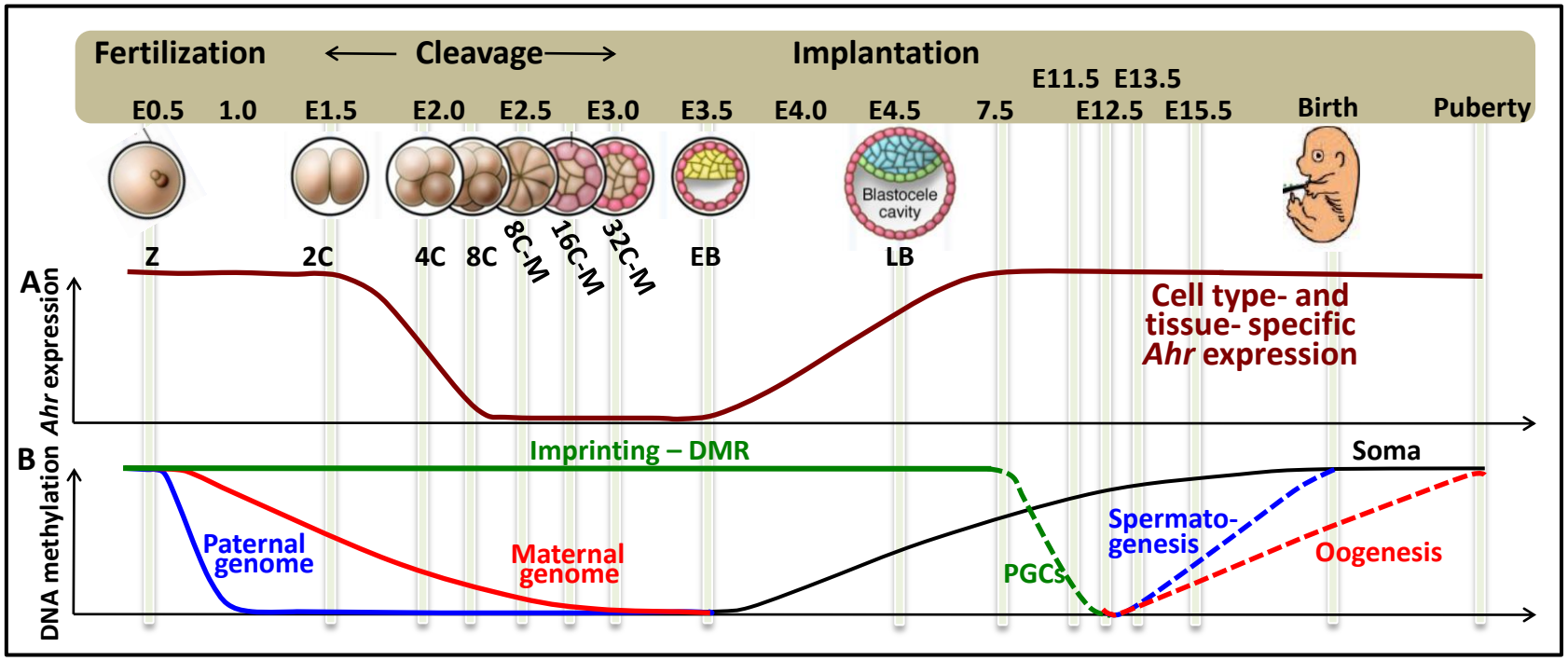
# Outline

- **AHR modulates lineage specification pathways during early differentiation**
  - ***Loss of pluripotency***
  - ***Cardiogenesis***
  - ***Neuroglia***

- **Dioxin exposure and *Ahr* ablation disrupt cardiogenesis**
  - ***Short and long-term effects of developmental dioxin exposure***

- **Gene-environment interactions in cardiac disease**
  - ***Ahr –Nkx2.5 interactions in cardiac haploinsufficiency***

# The preimplantation *Ahr* gene is silent

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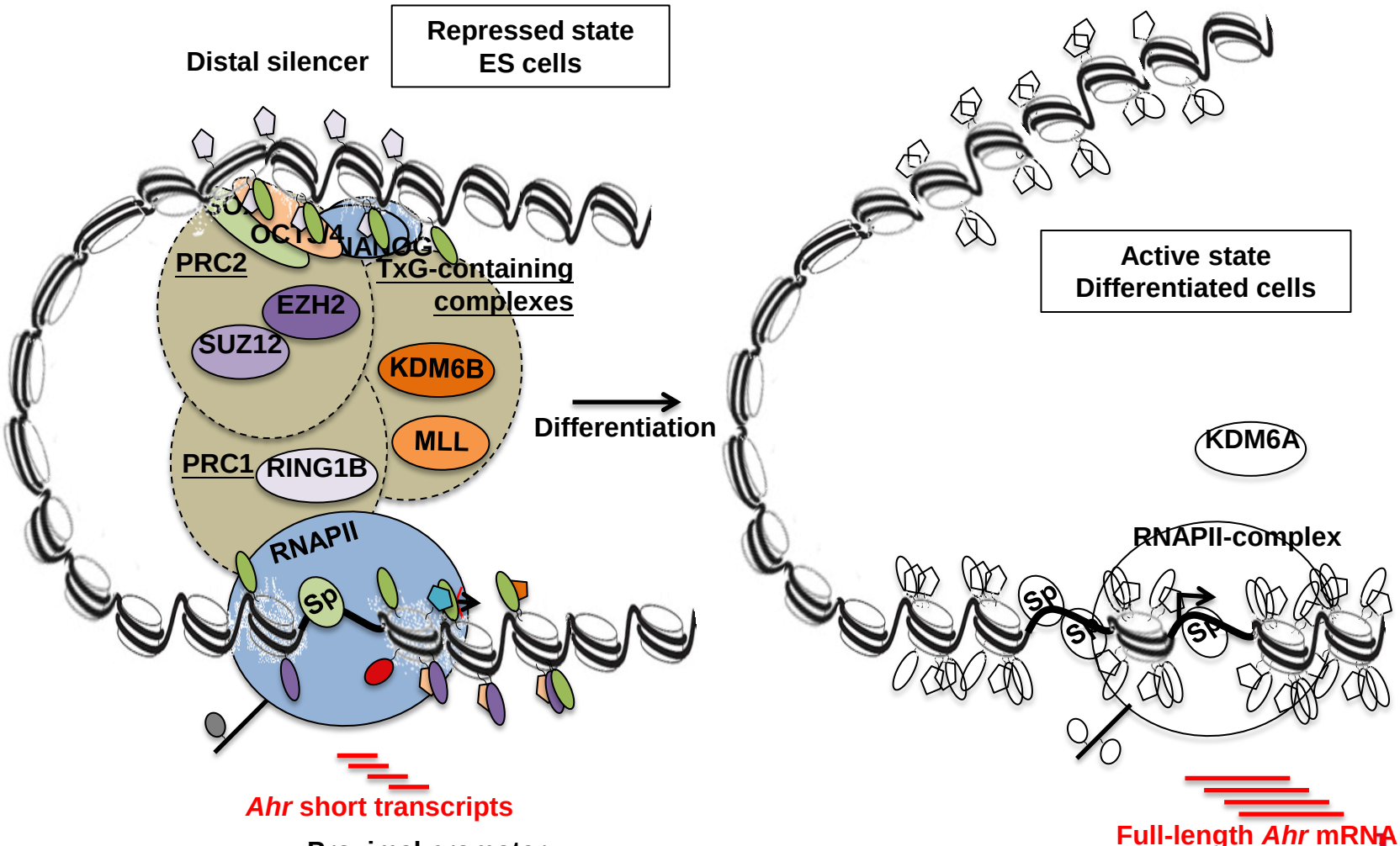
# The *Ahr* gene is repressed in ES cells




Ko, et al. Stem Cell Res. 12:296-308 (2013) polycomb/trithorax

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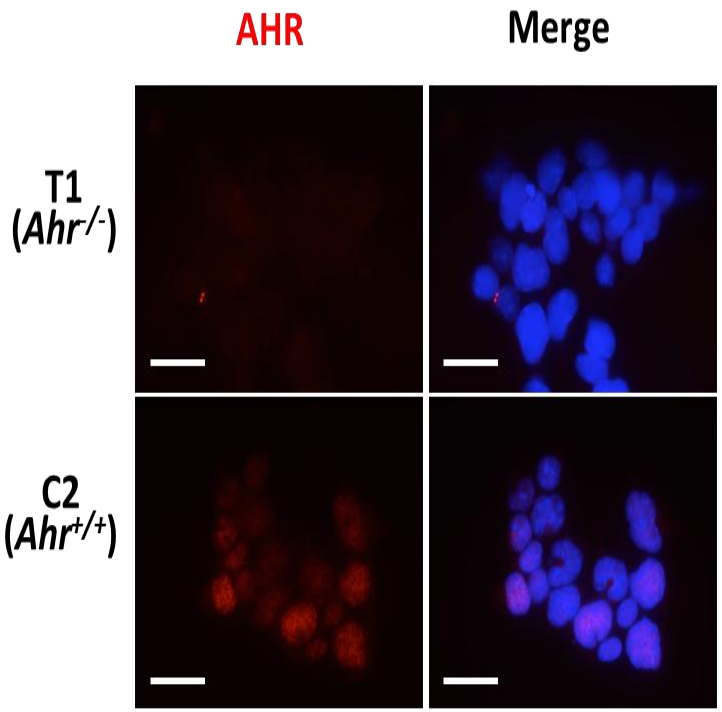
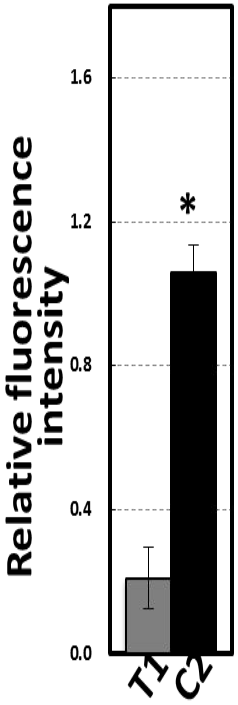
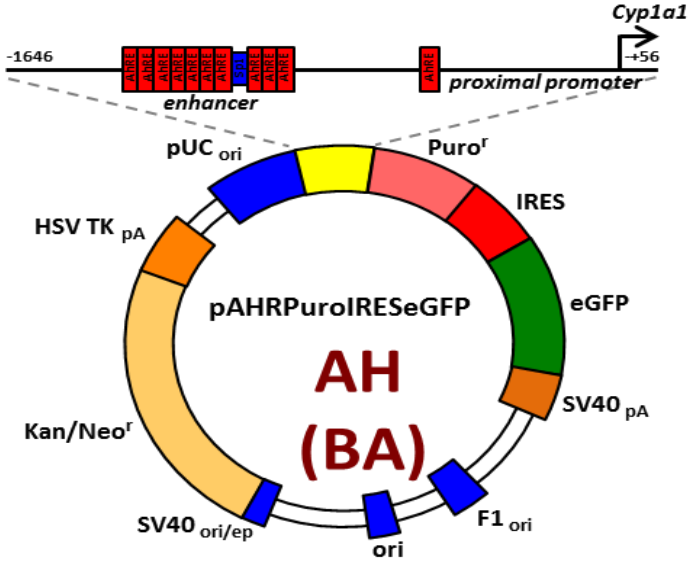
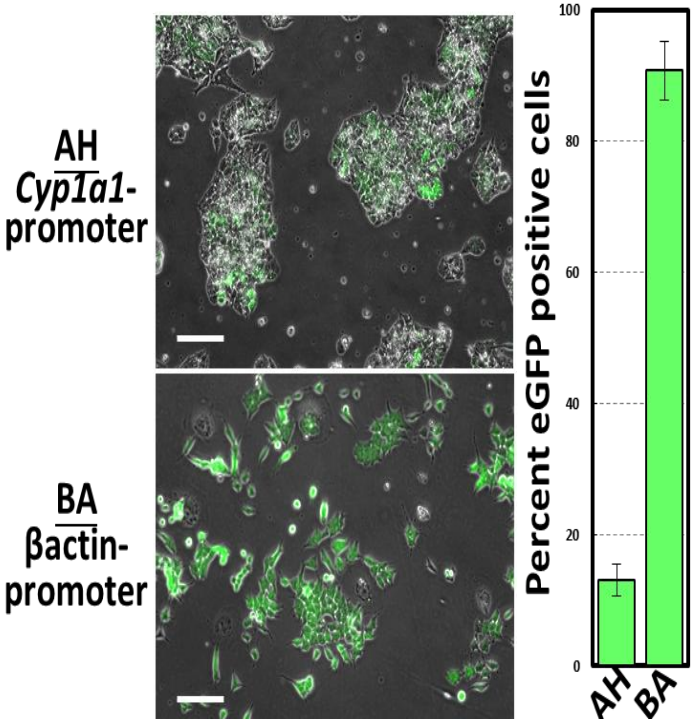
# 12 - 15% of the ES cell population expresses AHR

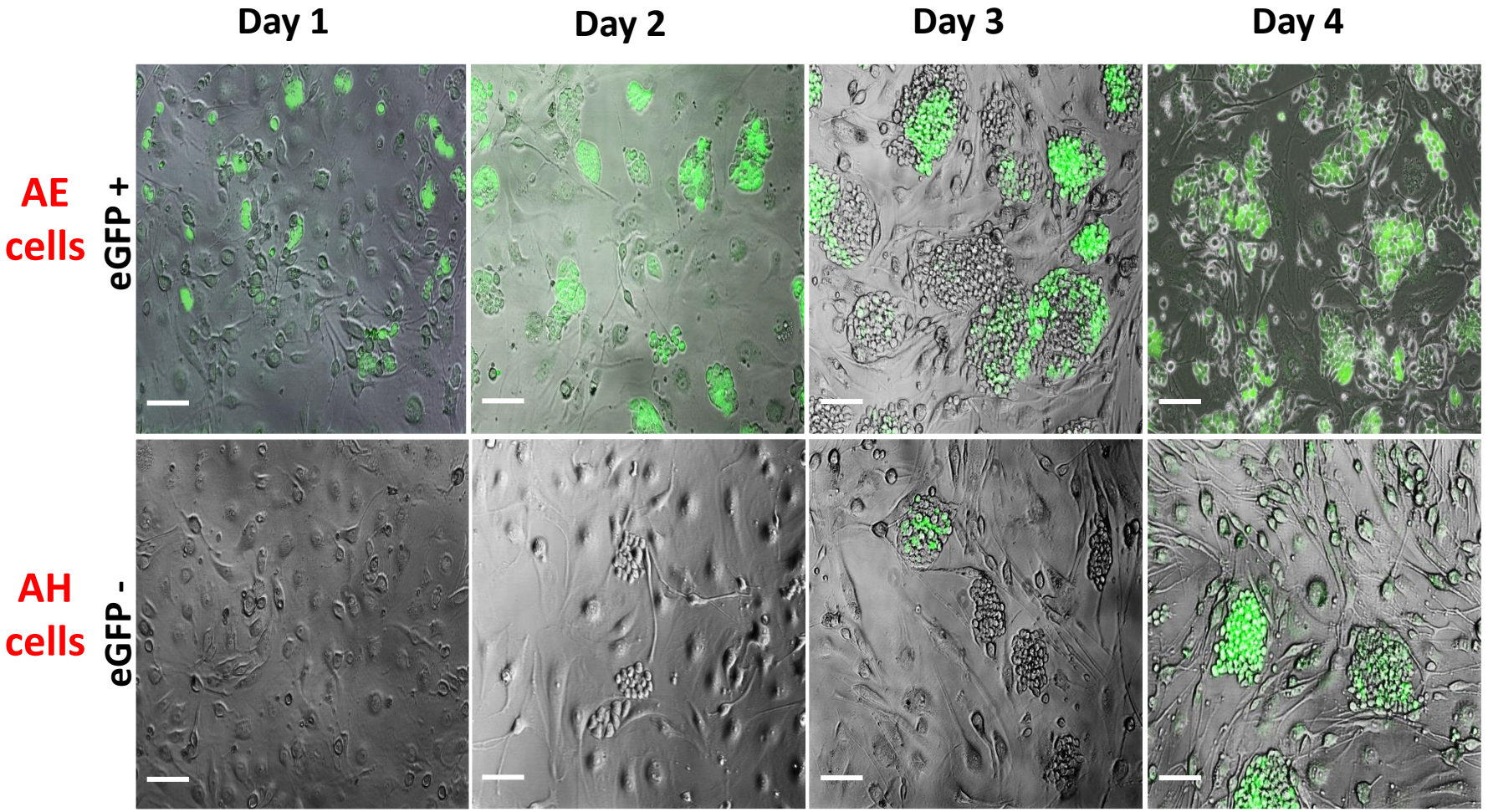


# Fluctuating state of AHR expression in ES cells

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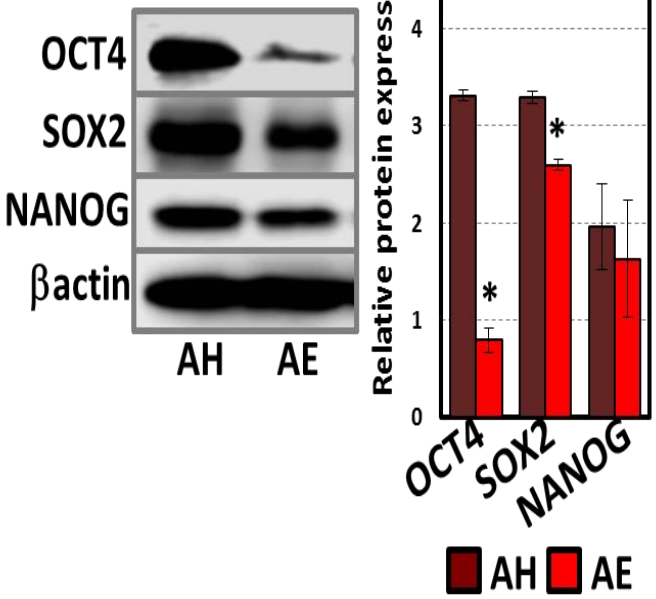
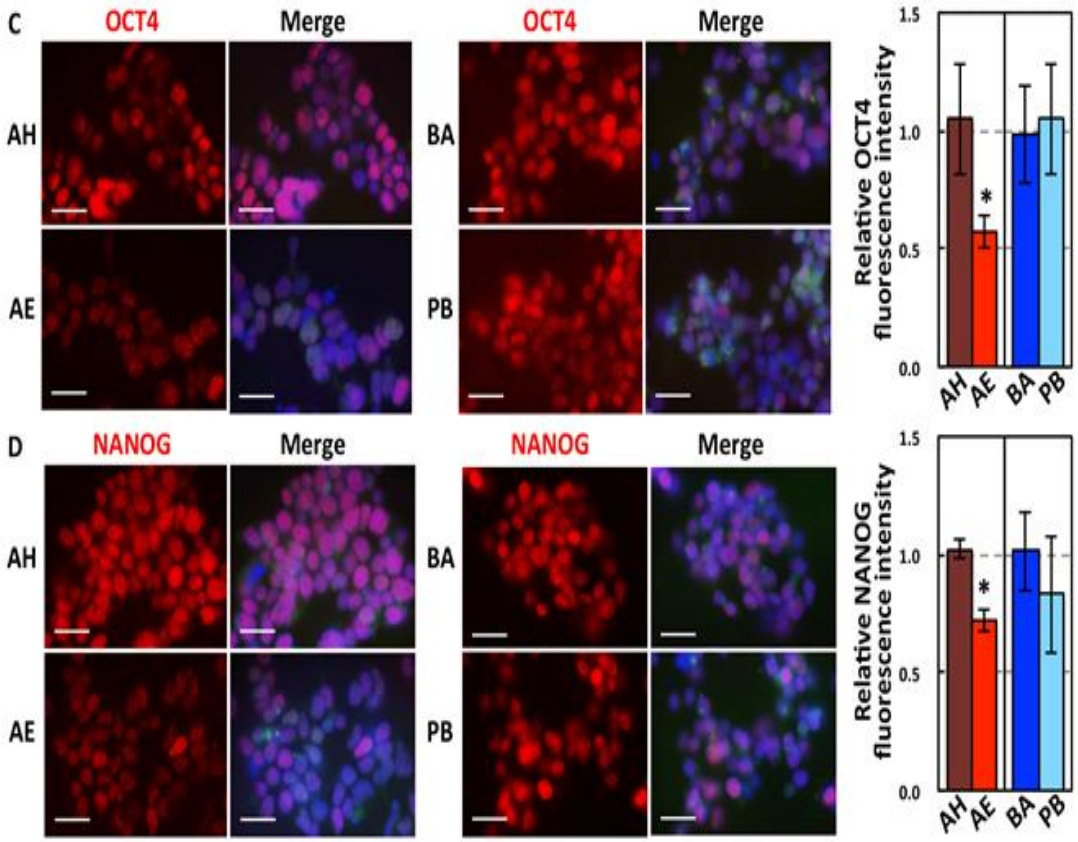
# Pluripotency factors are down-regulated in AE cells

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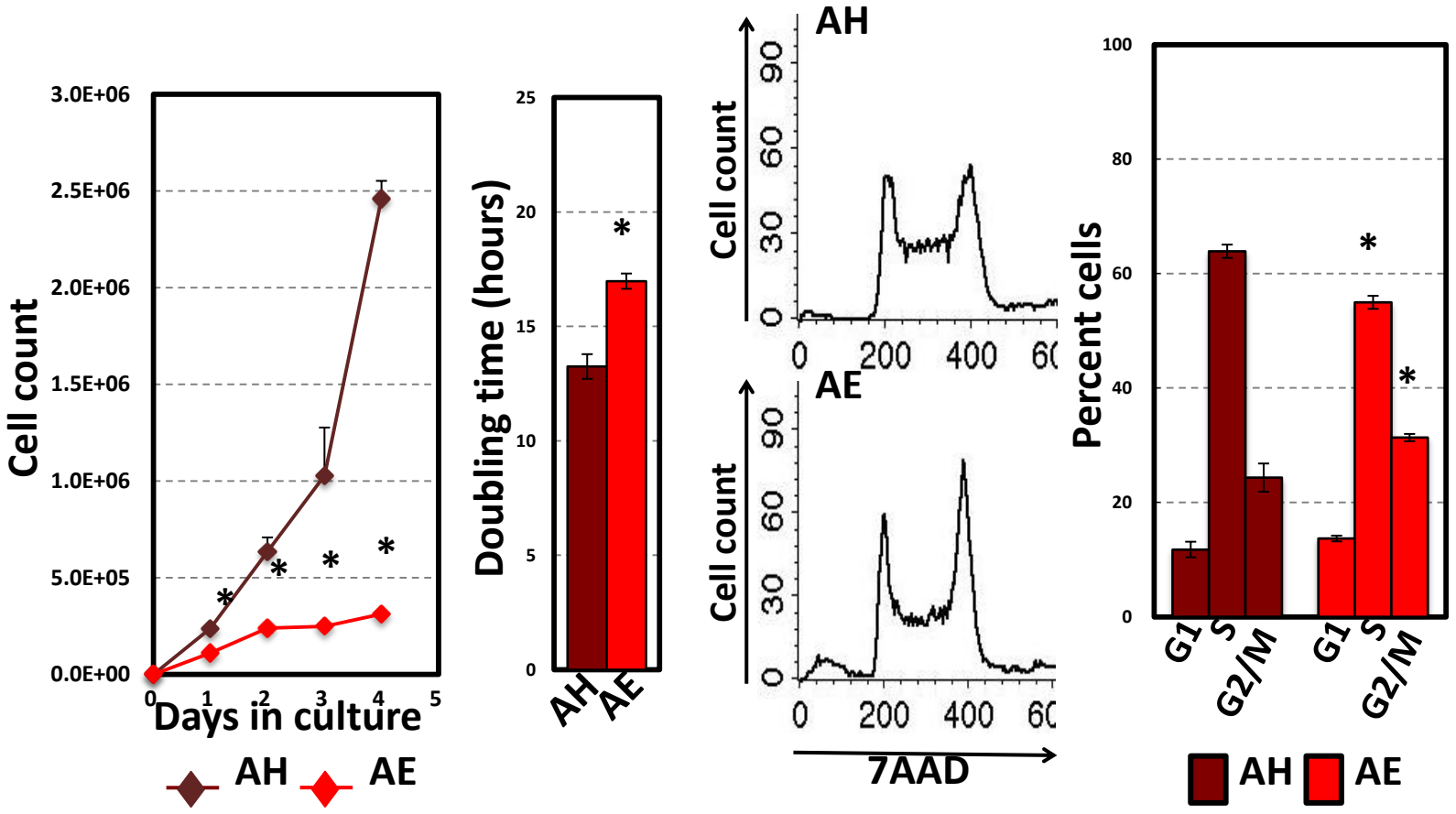
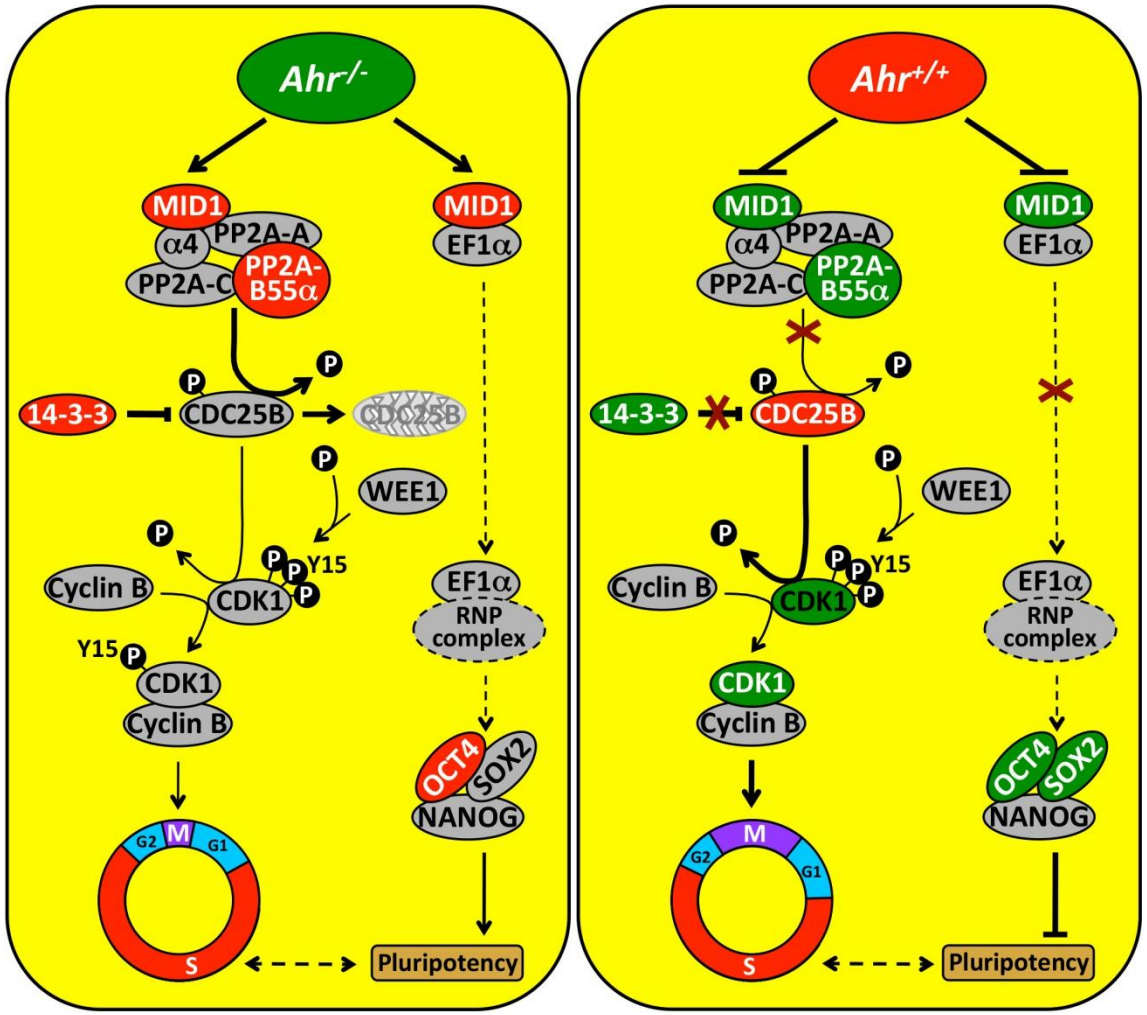
# AE cells proliferate more slowly and accumulate in G2/M



# AHR modulates mitotic progression and pluripotency




Ko, et al. Stem Cells. 2016 Jul 4. doi: 10.1002/stem.2456. [Epub ahead of print]

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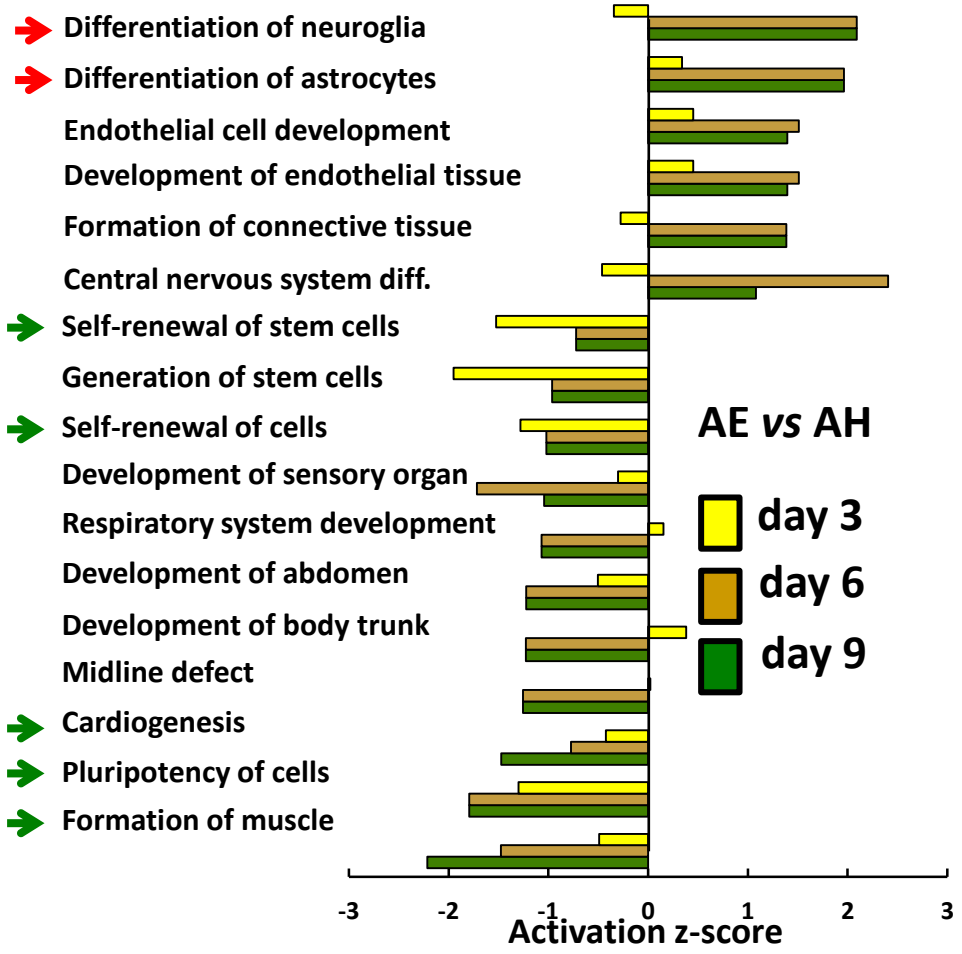
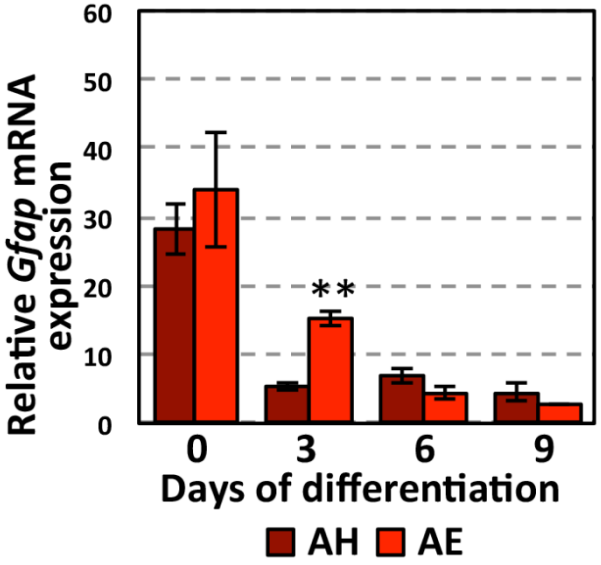
# AE cells promote unsustained neuroglia differentiation

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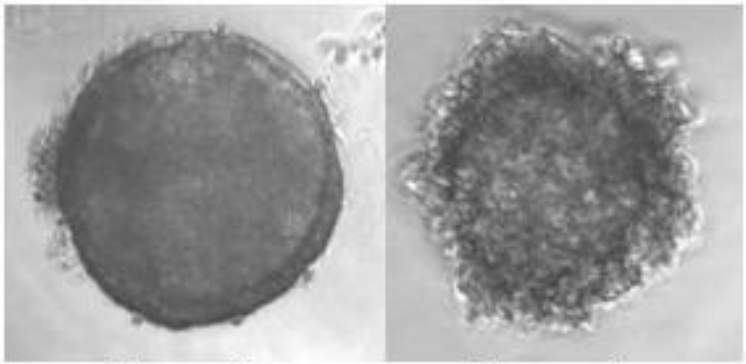
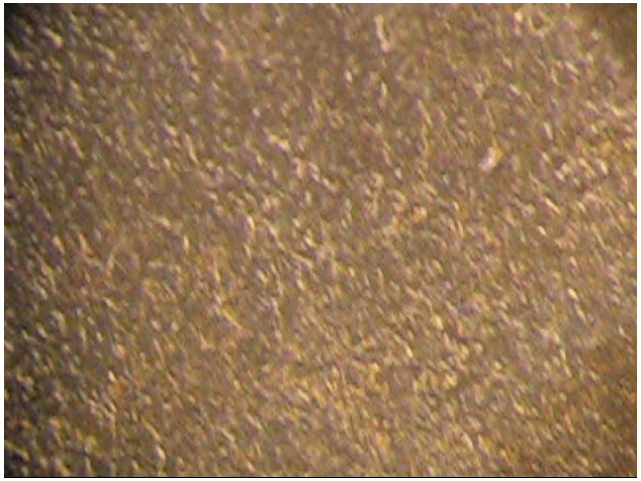
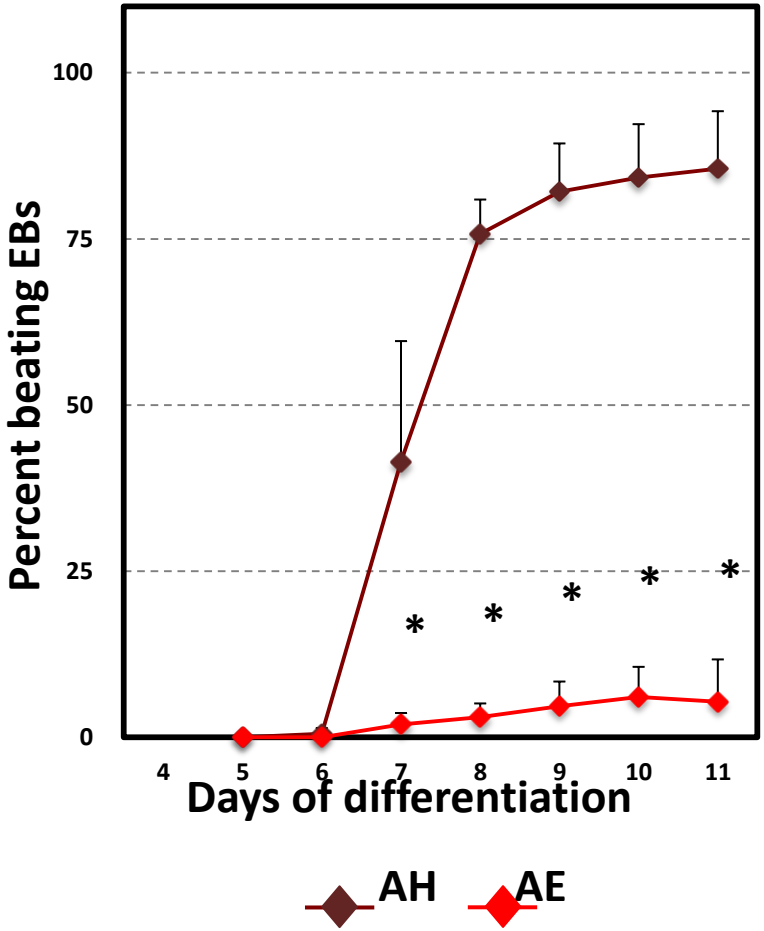
# Mouse ES cells form embryoid bodies




# that differentiate into beating cardiomyocytes



# Not the embryoid bodies formed by AE cells

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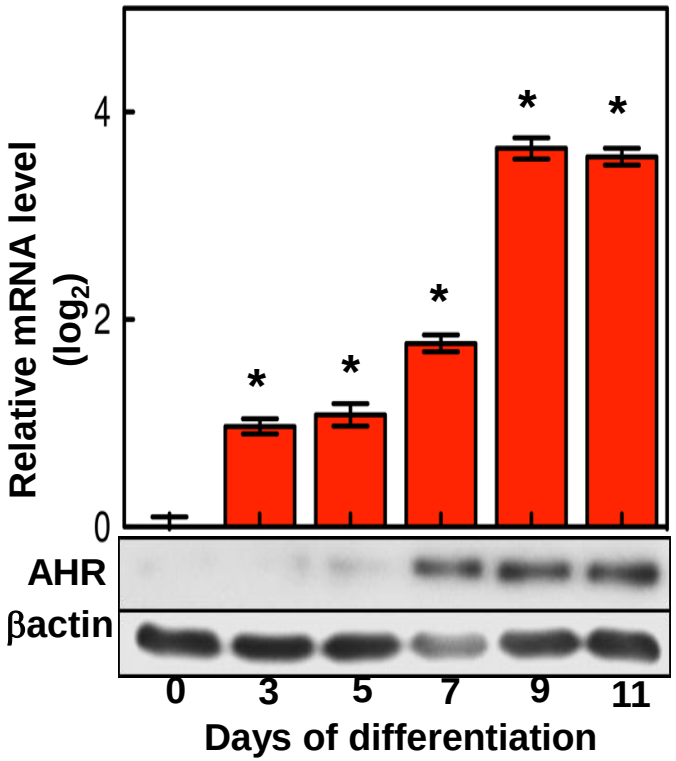
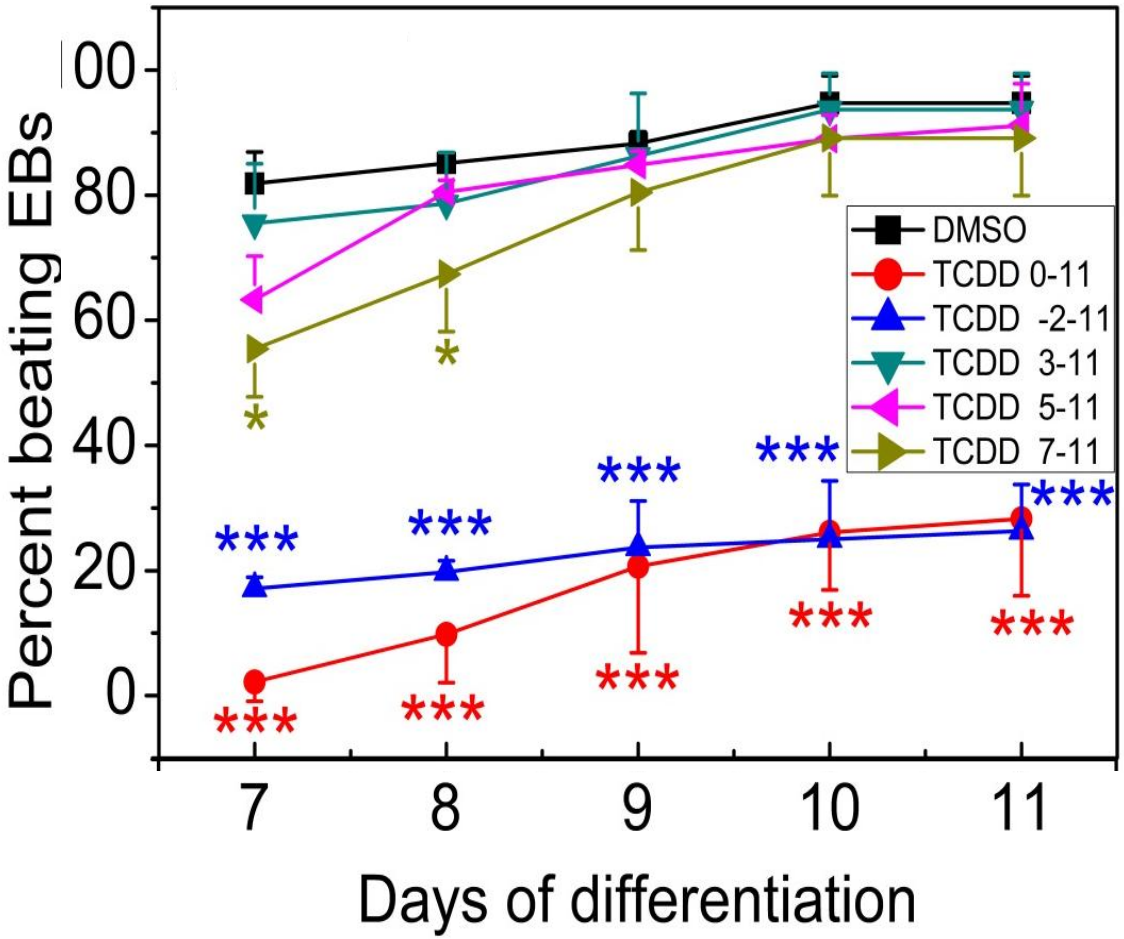
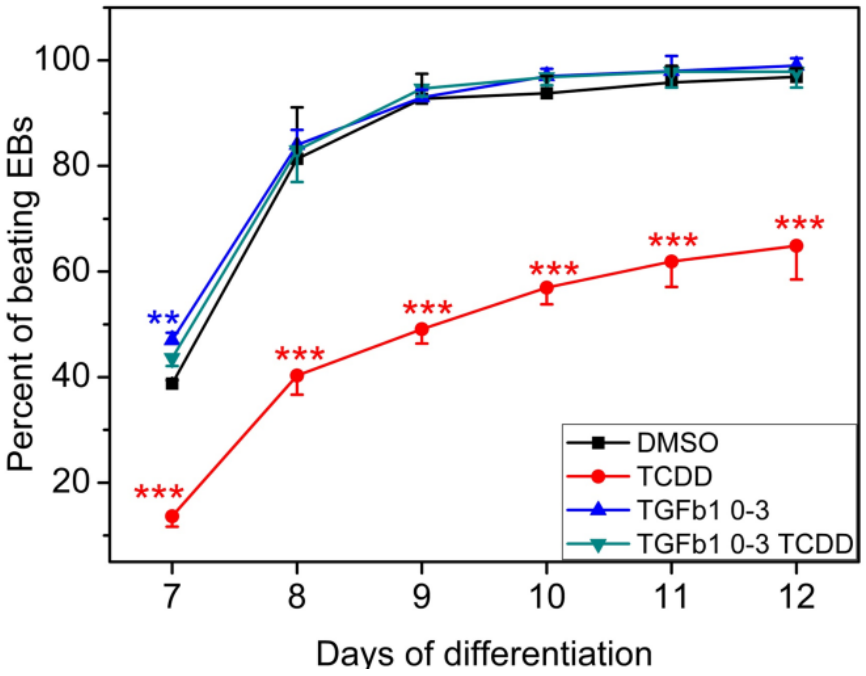
# *Ahr* expression is up-regulated as ES cells differentiate

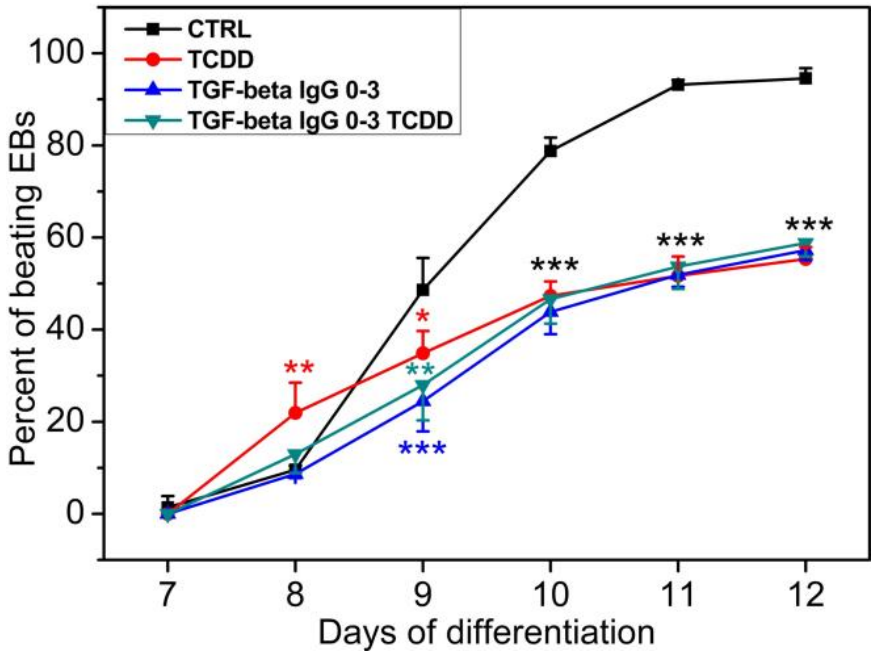


# Cardiomyocyte differentiation has a narrow window of TCDD sensitivity

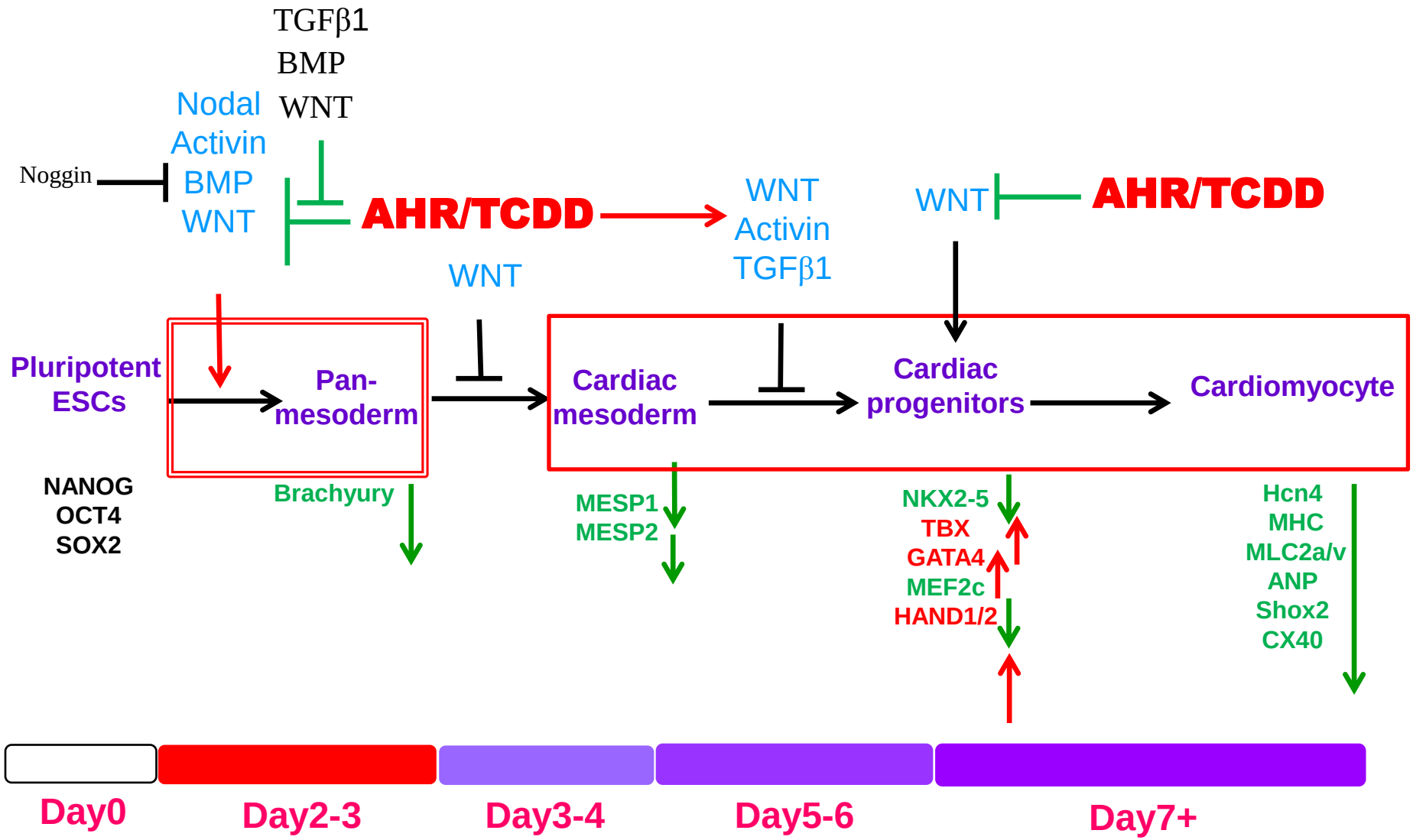


# TGFβ1 restores contractility blocked by TCDD



# The AHR/TCDD axis interferes at multiple developmental stages with the differentiation of contractile cardiomyocytes



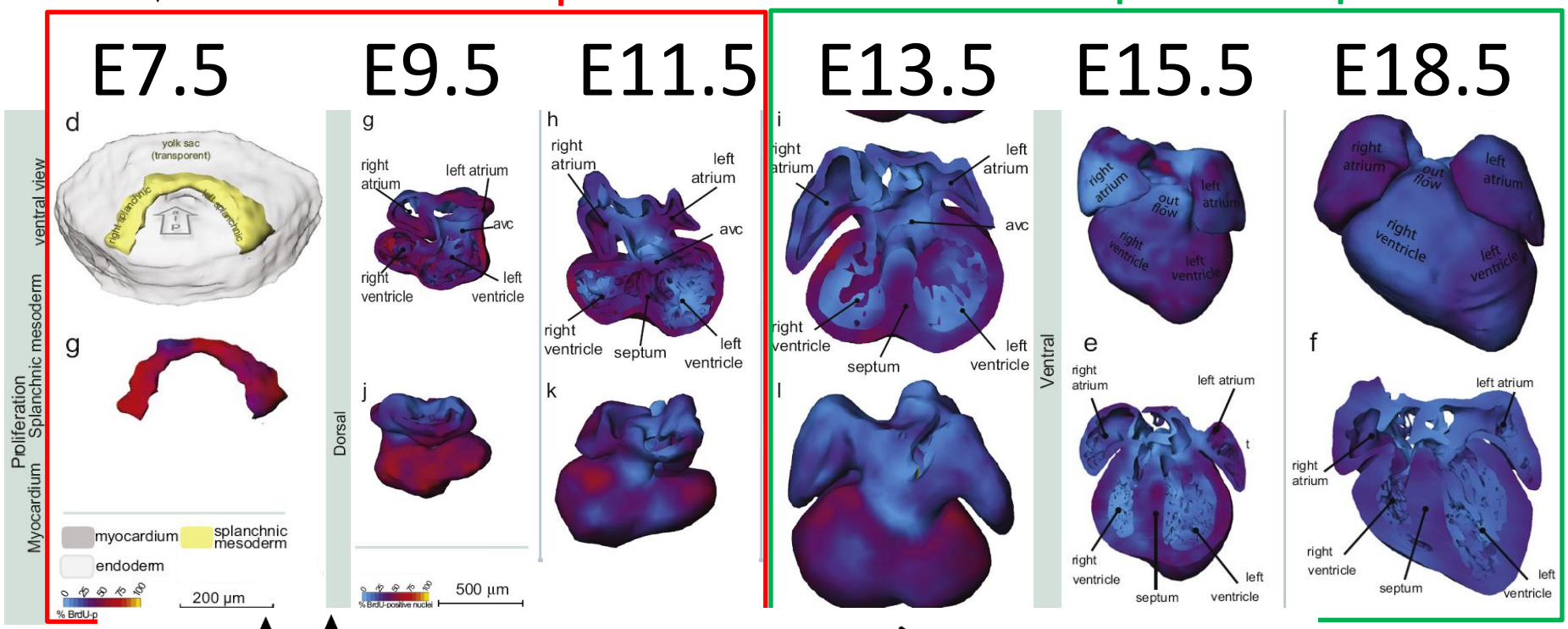
First organ

# Dosing scheme—*in vivo* analyses

Organogenesis

**TCDD exposure**

**Developmental Samples**




Modeling (septation)

Beating

Pacemaker

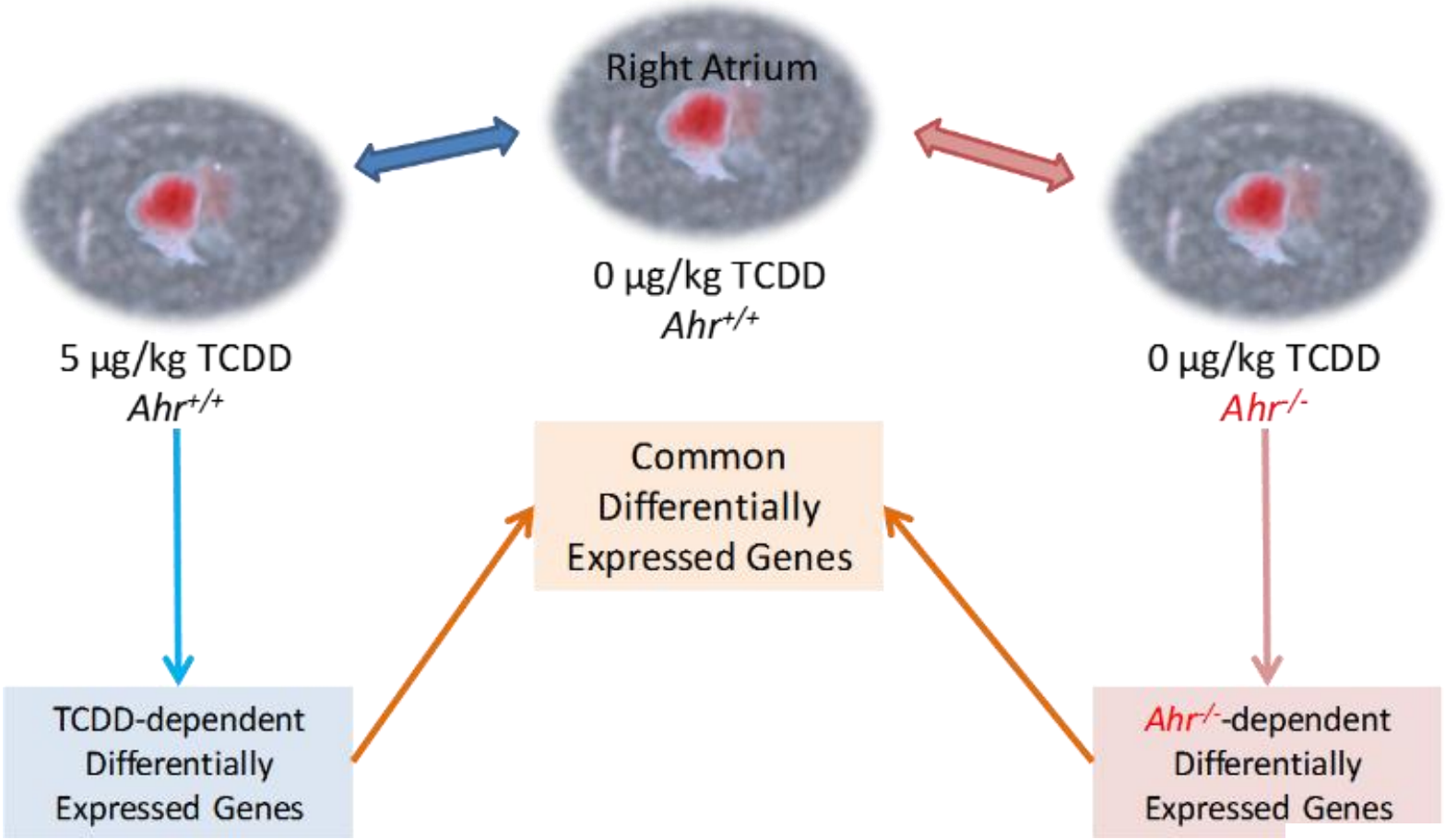
Remodeling (valves, compaction)

Myocyte proliferation

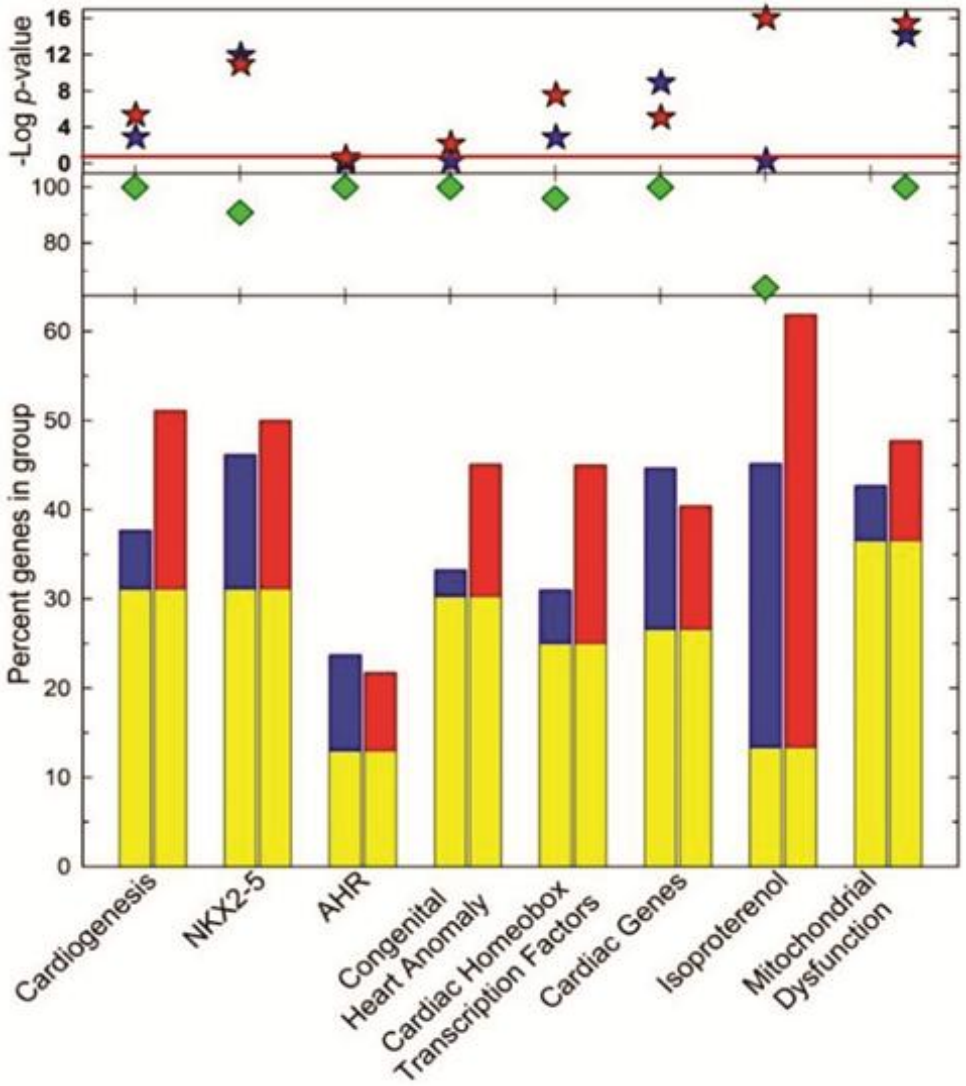
Myocyte maturation

De Boer et al. 2012

# What are the gene expression effects of AHR disruption?



# AHR disruption affects the expression of key networks involved in cardiogenesis, cardiac homeostasis, and mitochondrial function




TCDD at E9.5 0 - E11.5 RNA.seq right atrium at E15.5

TCDD

*Ahr*$^{-/-}$

Common

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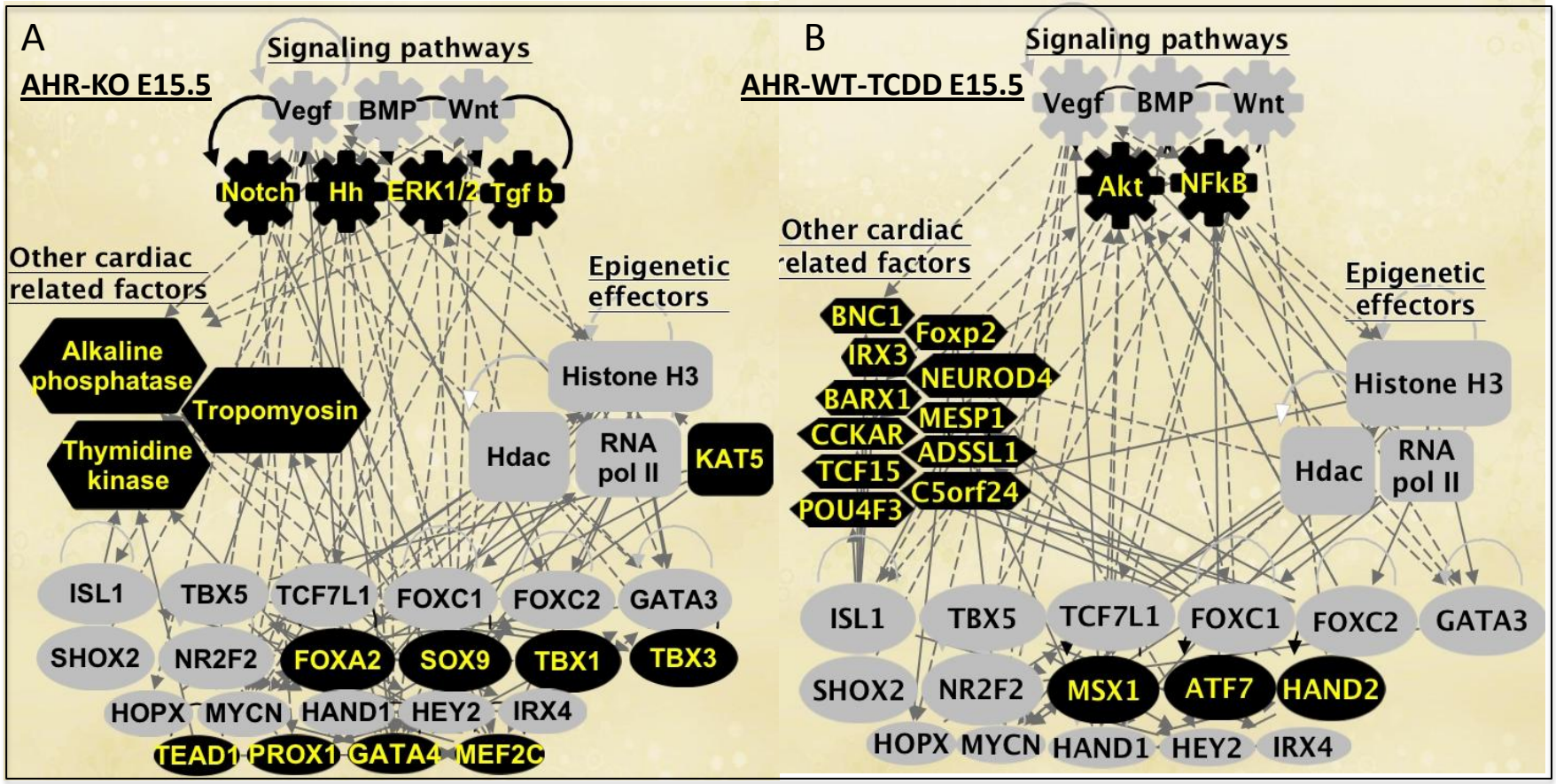
# Gene networks common and not-common to *Ahr* KO and TCDD exposure

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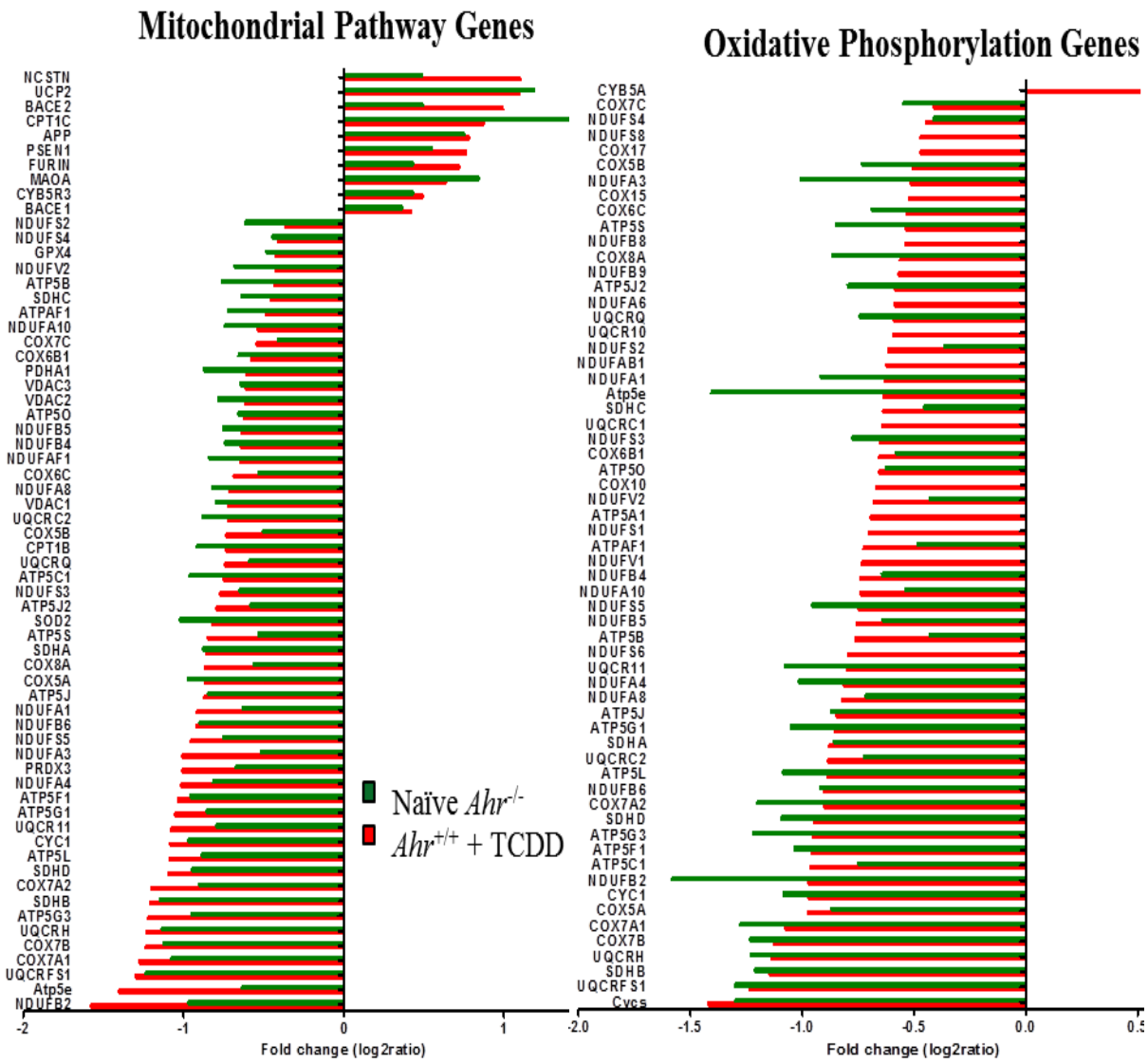
# Genes deregulated at E15.5

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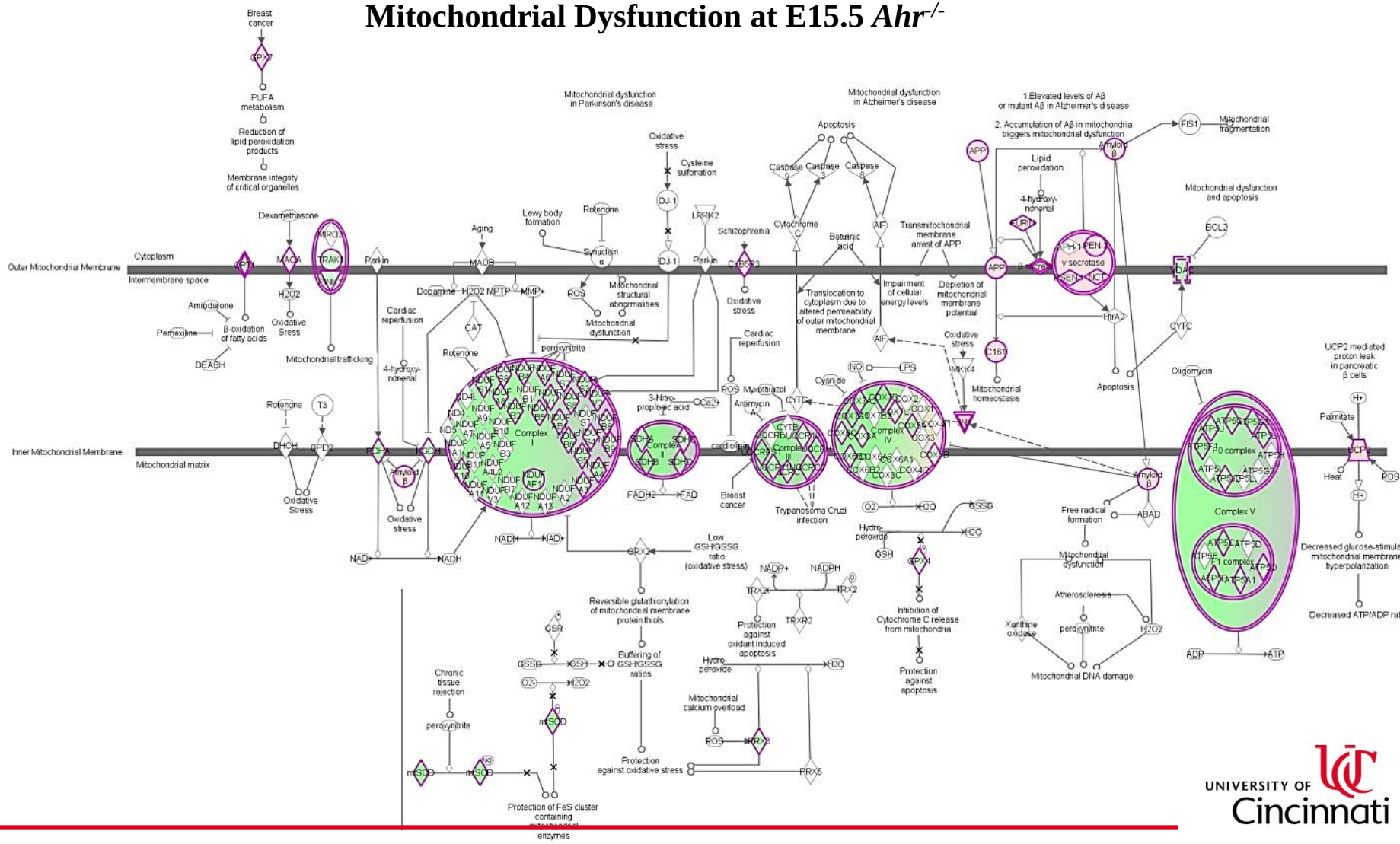
# Developmental AHR disruption globally downregulates mitochondrial complexes

**Mitochondrial Dysfunction at E15.5 *Ahr*$^{-/-}$**

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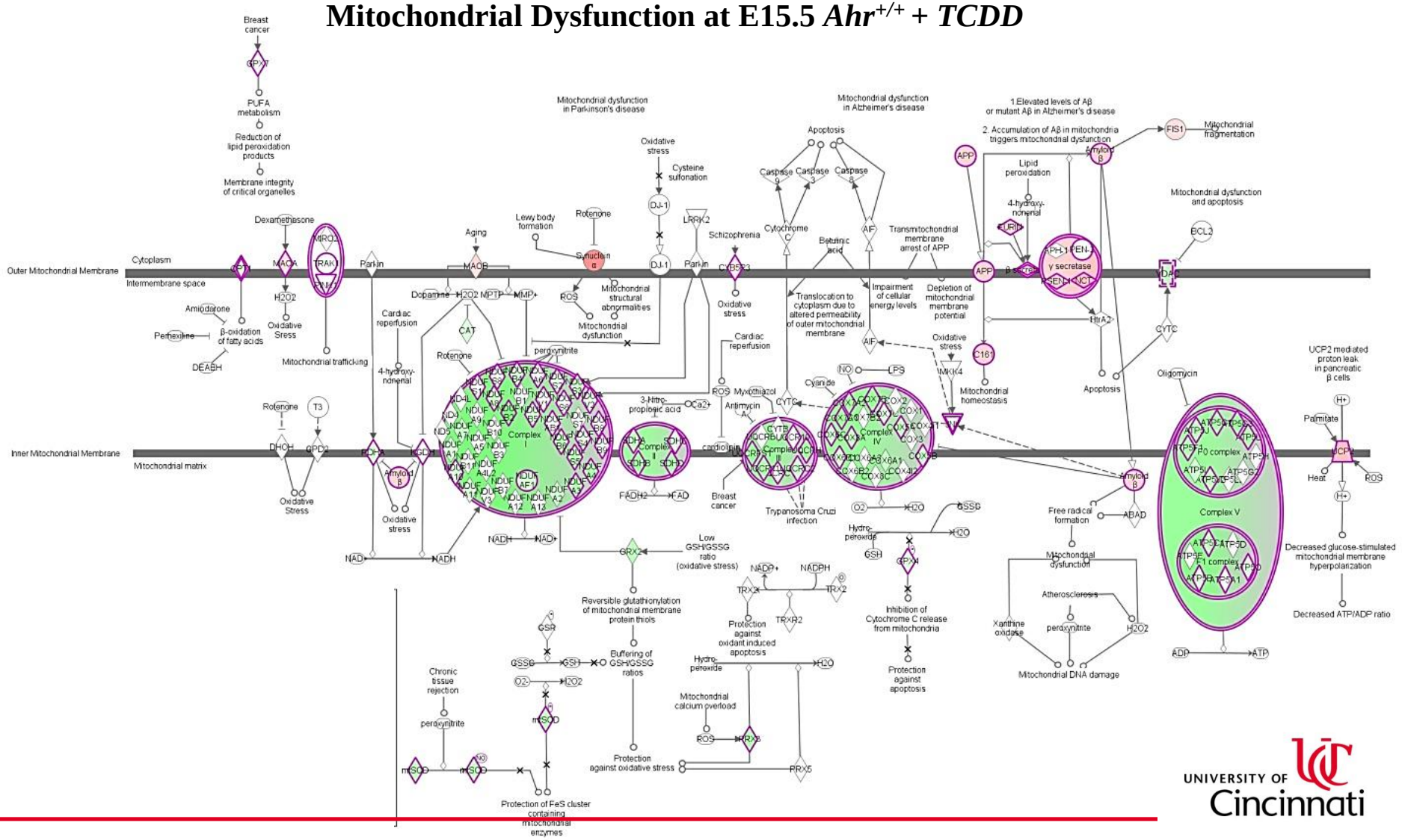
# Developmental AHR disruption globally downregulates mitochondrial complexes

**Mitochondrial Dysfunction at E15.5 *Ahr*$^{+/+}$ + *TCDD***

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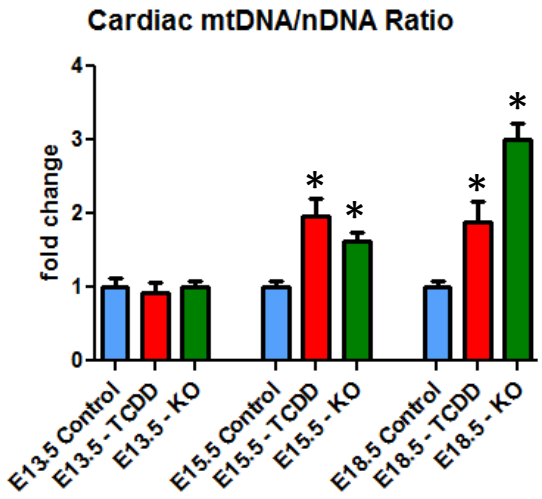
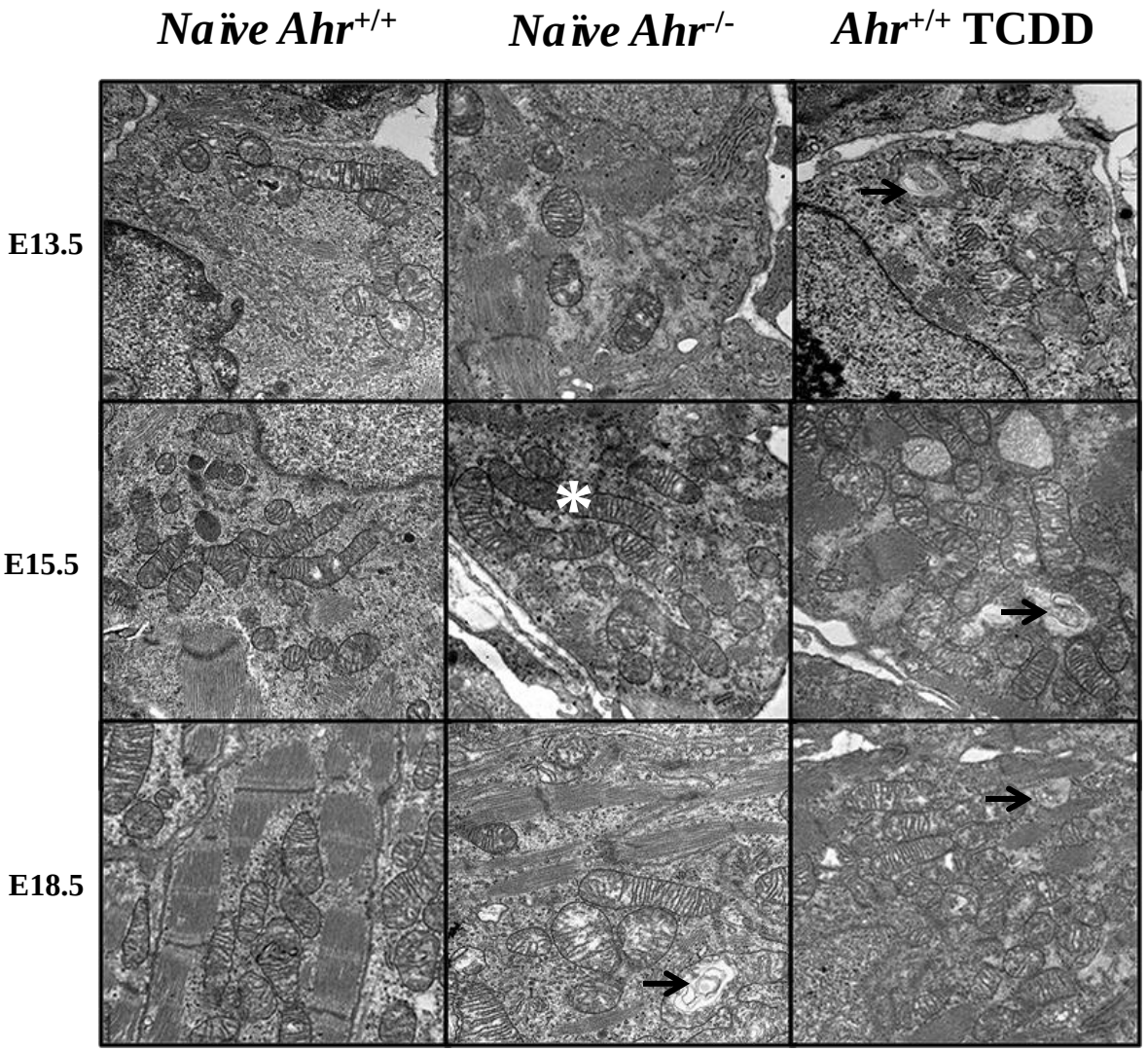
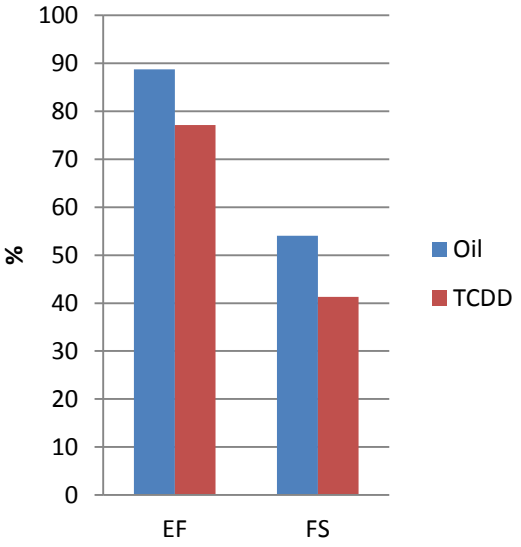
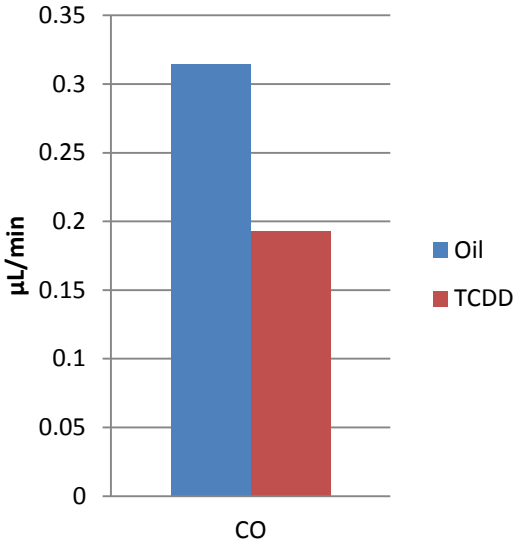
# Developmental AHR disruption affects mitochondria structure and abundance

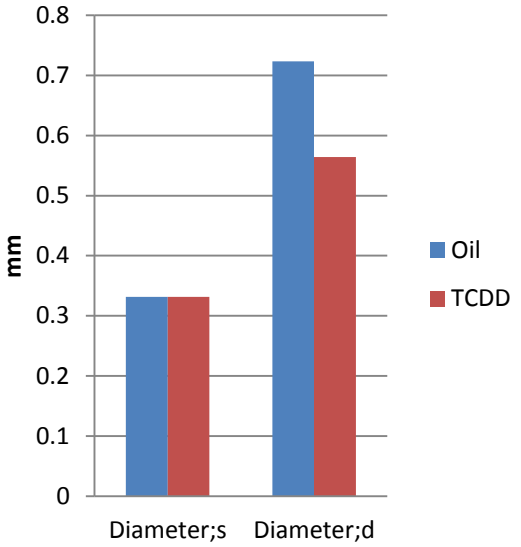


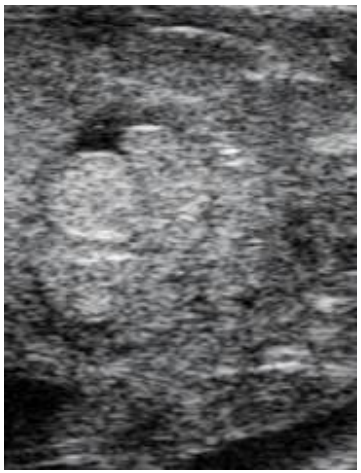



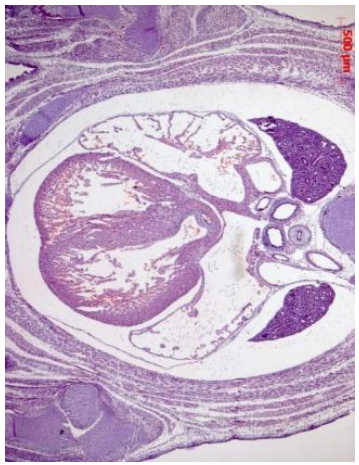
# Decline of cardiovascular performance in TCDD-exposed E15.5 embryos determined by very high frequency echosonography

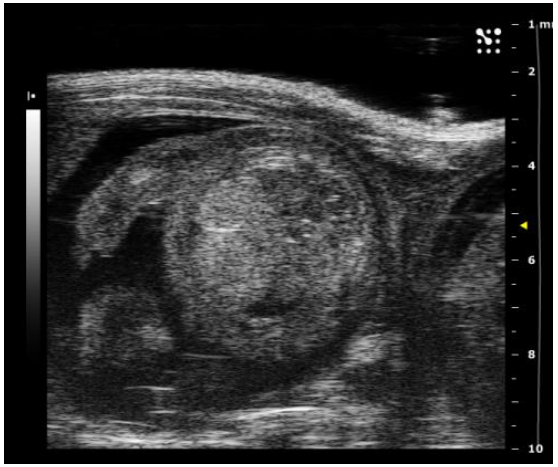






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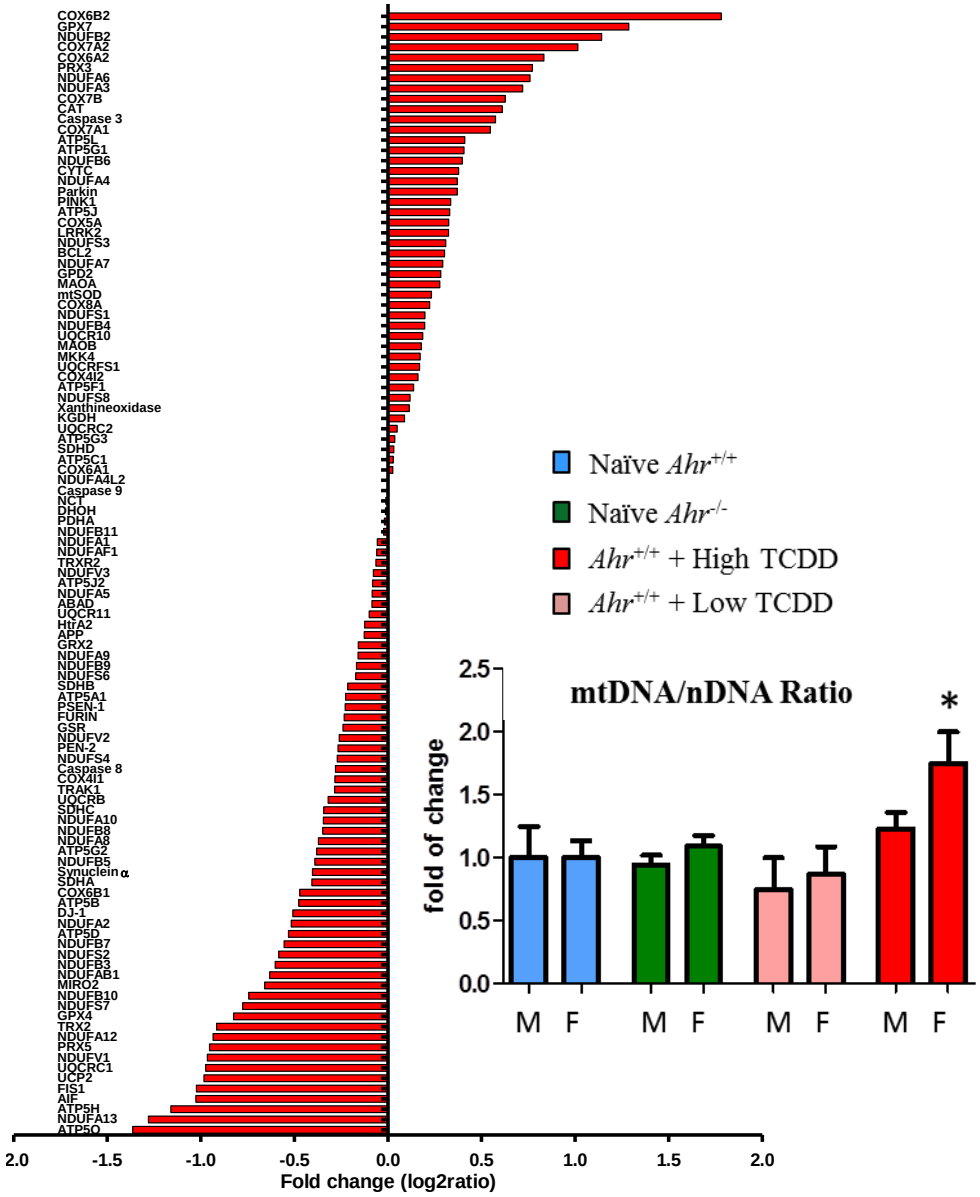
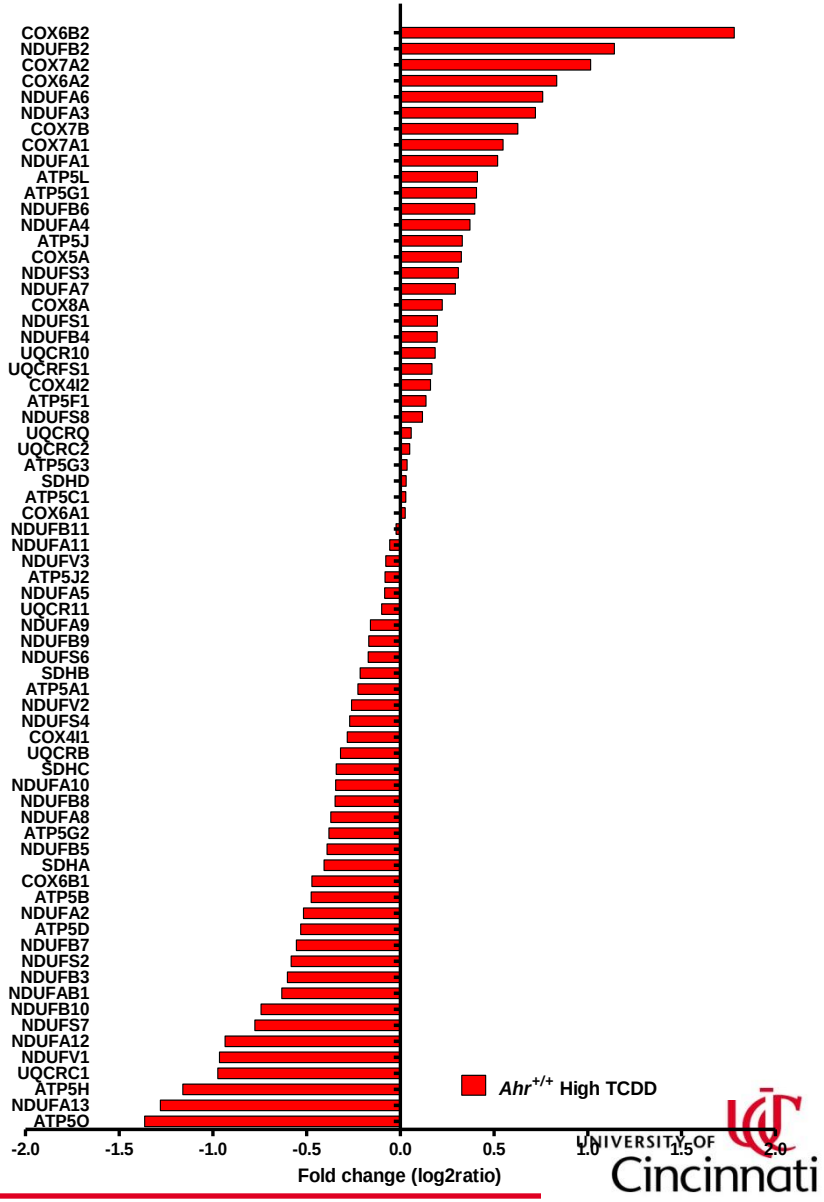
# Gene expression changes remain 9 months later

## Mitochondrial Pathways Genes




## Oxidative Phosphorylation Genes

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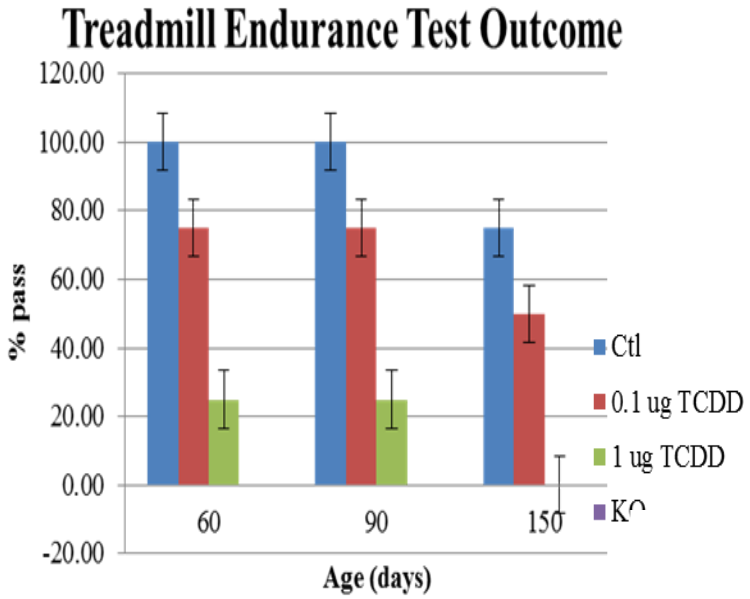
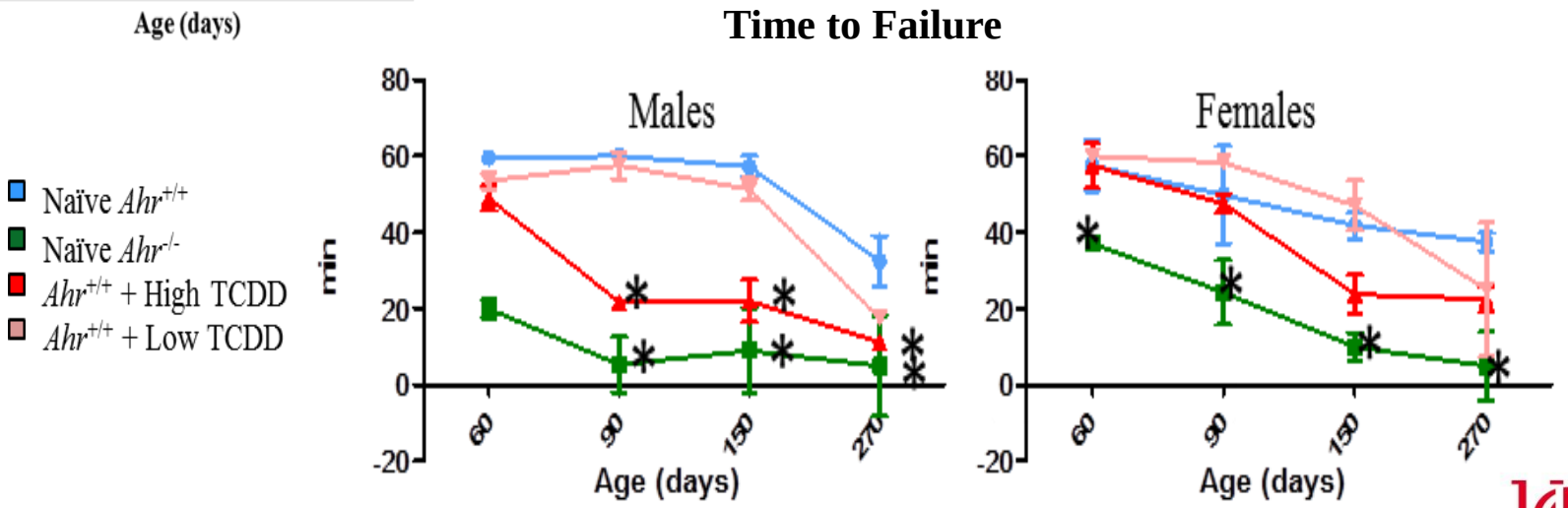
# Treadmill endurance test of *Ahr-/-* and *Ahr+/+* mice exposed or not to TCDD *in utero*

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# The molecular signature of developmental AHR disruption is similar to the *Nkx2-5*$^{-/-}$ heart transcriptome

| *Nkx2-5*$^{-/-}$ gene expression (*Prall et al 2007*) | *Ahr*$^{+/+}$ + TCDD | | *Naïve Ahr*$^{-/-}$ | |
|---|---|---|---|---|
| **Downregulated** | **Direction** | **Agrees Nkx2-5 mutant** | **Direction** | **Agrees Nkx2-5 mutant** |
| Ankrd1 | up | no | unchanged | no |
| Nppb (Bnp) | down | yes | down | yes |
| Nr2f2 (coup TFII) | up | no | up | no |
| Myl2 | down | yes | down | yes |
| Irx4 | down | yes | down | yes |
| Smpx (Chisel) | unchanged | no | down | yes |
| Nppa (Anf) | up | no | up | no |
| Wnt11 | up | no | up | no |
| **Upregulated** | | | | |
| IGFBP5 | up | yes | up | yes |
| Pdgfra | up | yes | up | yes |
| Tnc | up | yes | up | yes |
| Isl1 | up | yes | up | yes |
| Tbx5 | up | yes | up | yes |
| Bmp2 | unchanged | no | unchanged | no |
| Fgf10 | up | yes | up | yes |
| Hhex | unchanged | no | unchanged | no |
| **Unaltered** | | | | |
| Cer1 | unchanged | yes | unchanged | yes |
| Dan | unchanged | yes | unchanged | yes |
| NOG | unchanged | yes | unchanged | yes |
| Bmp4 | up | no | up | no |
| Bmp7 | down | no | down | no |
| Dkk1 | unchanged | yes | unchanged | yes |

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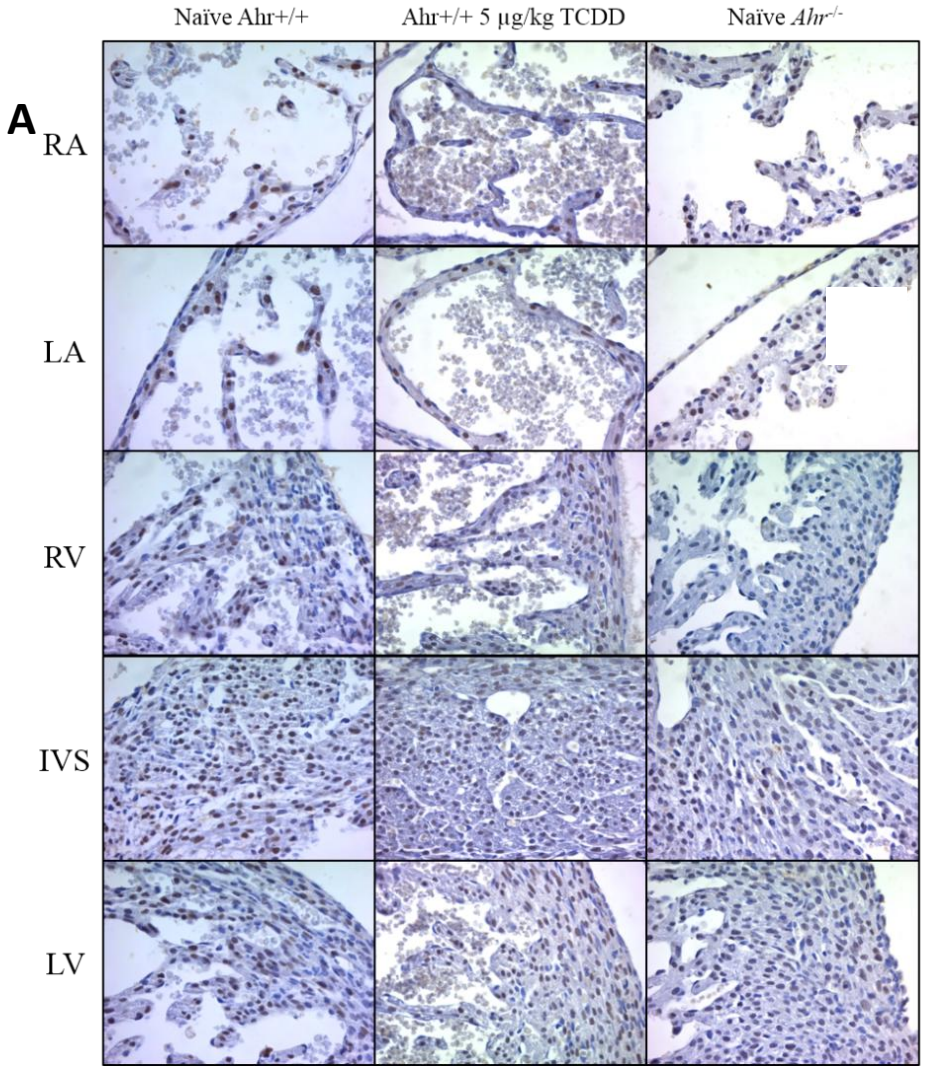
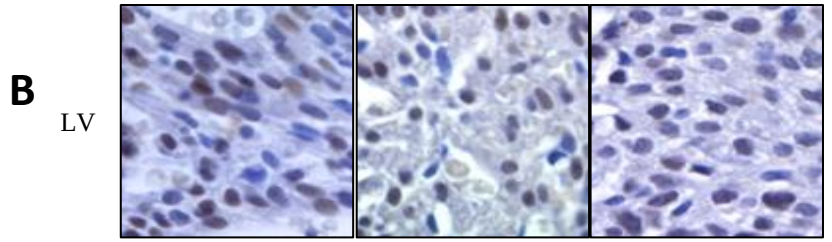
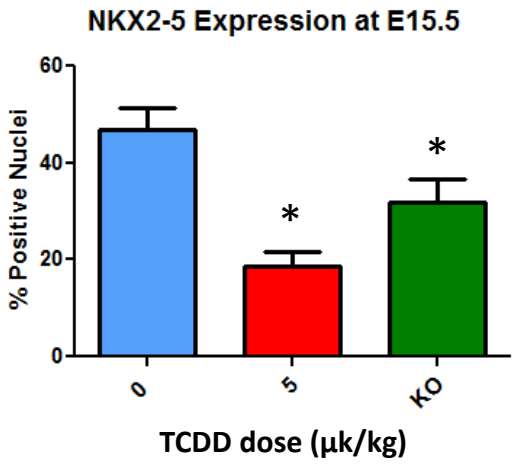
# Loss of *Ahr* or TCDD exposure repress NKX2-5




B

C

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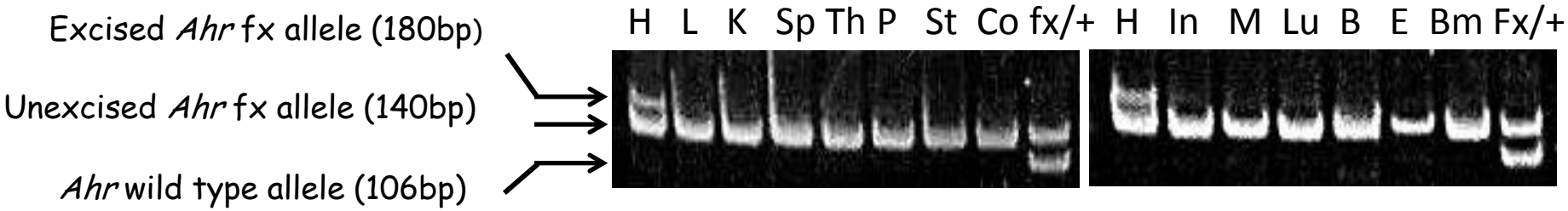
# Heart-specific *Ahr* knockout with a *Nkx2-5*$^{+/cre}$ knockin




**The genotype of these mice is *Nkx2-5*$^{+/cre}$ *Ahr*$^{fx/fx}$**

**Because they have only one functional *Nkx2-5* allele**
**<u>These mice are haploinsufficient</u>**

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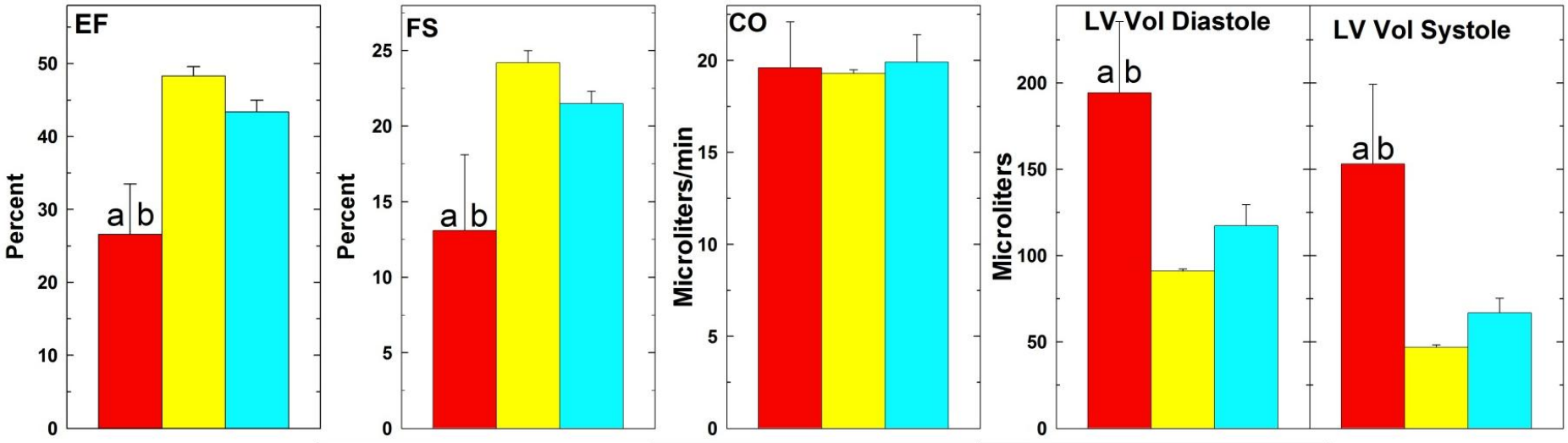
# *Ahr* ablation rescues NKX2.5-dependent cardiac insufficiency




***Nkx2.5*** **haploinsufficient**
***Ahr*** **wild-type**

***Nkx2.5*** **wild-type**
***Ahr*** **floxed (wild-type)**

***Nkx2.5*** **haploinsufficient**
***Ahr*** **excised**

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# Congenital Cardiac Disease

*NKX2.5* ⟵ *AHR* ⟶ Environment

Congenital cardiac malformations
Valvular stenosis
Hypoplastic left heart syndrome
8 per 1000 live births
25 – 30% of cardiac disease
Deadly if not surgically repaired at birth

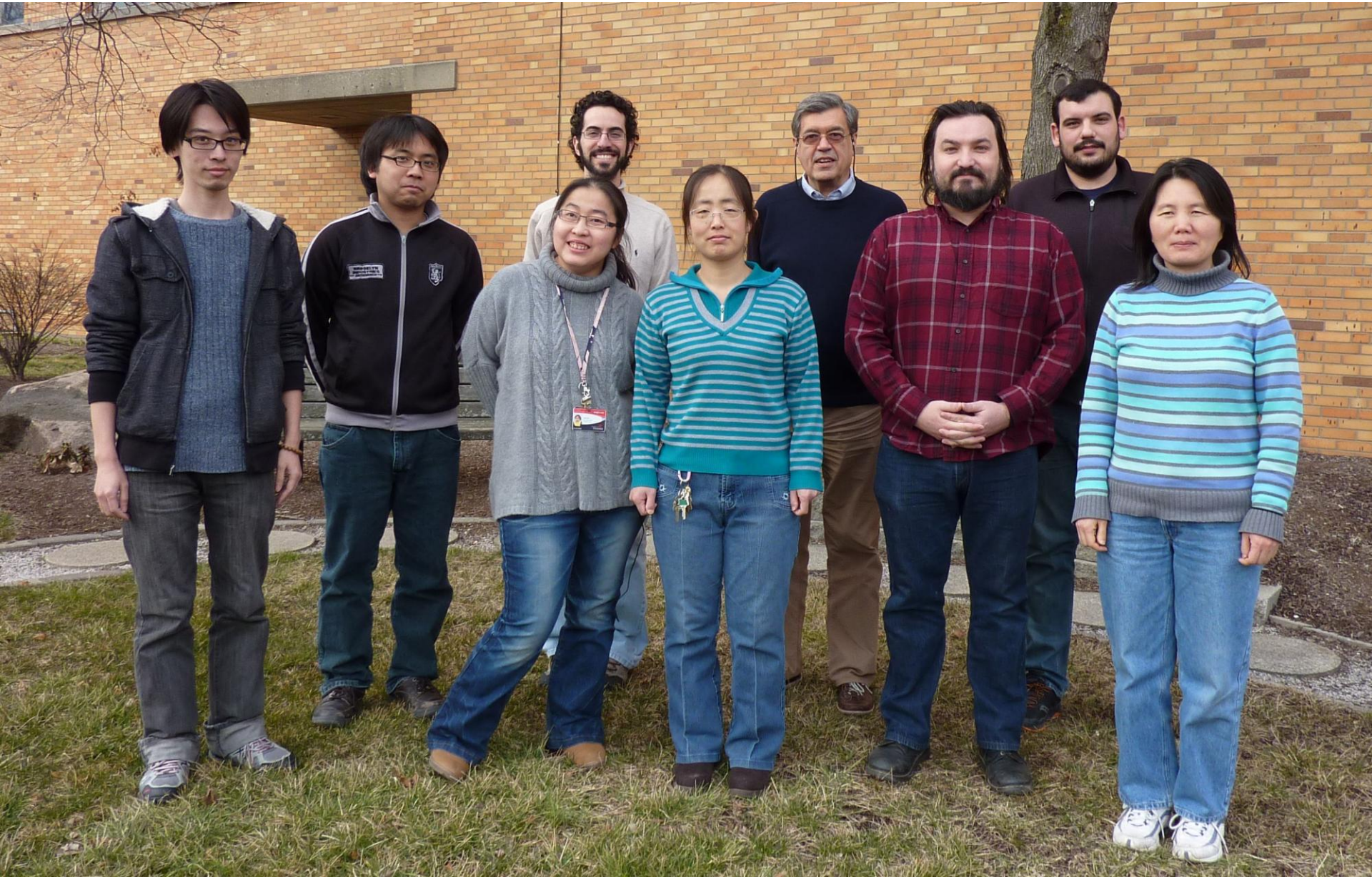
# I hope that I have convinced you that …

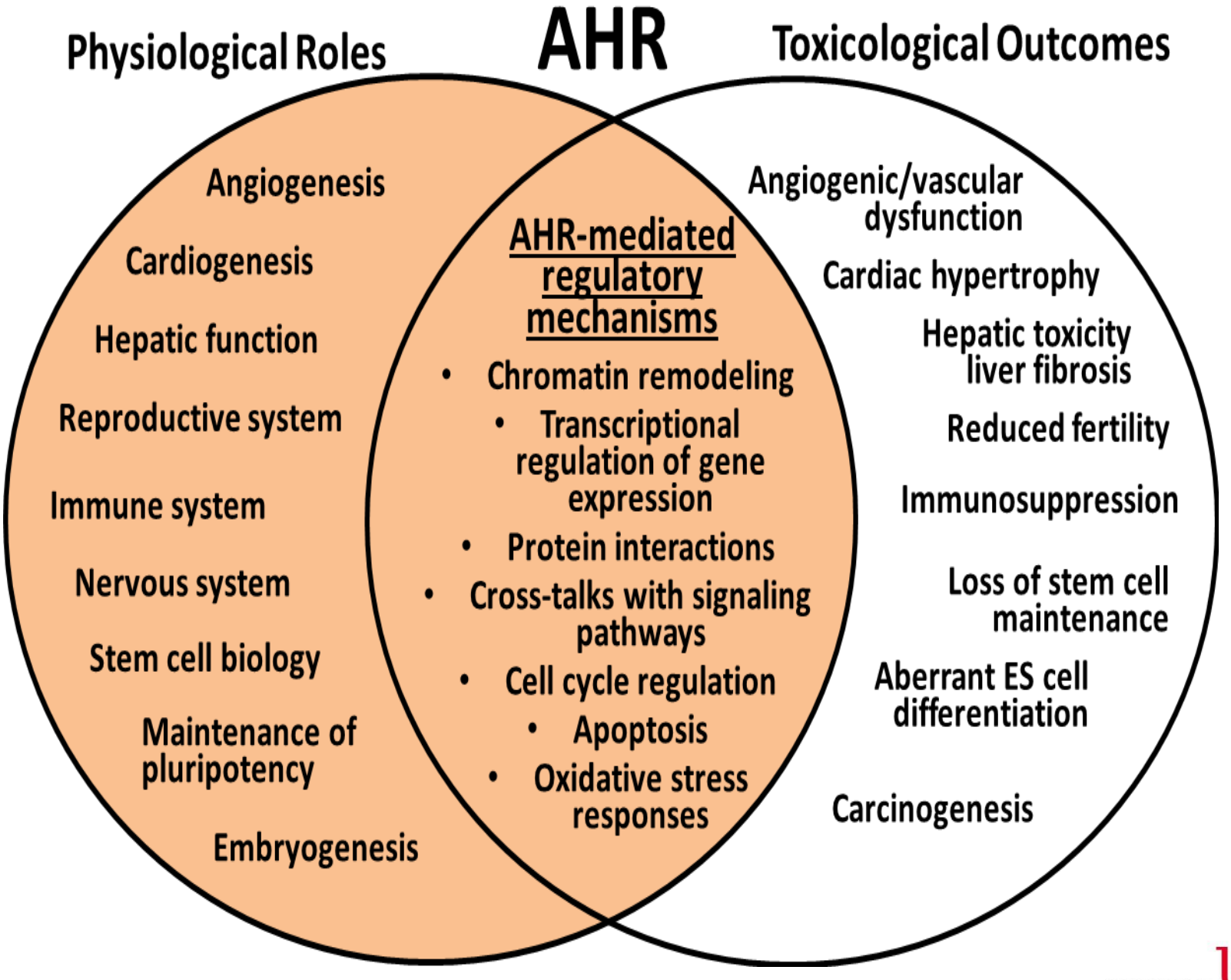
**The *Ahr* gene is positively silenced in ES cells**

***Ahr* modulates lineage specification pathways during early differentiation**

***Ahr* ablation and dioxin exposure disrupt cardiomyocyte differentiation**
***Gene networks, Mitochondria, OXPHOS, Heart function, Endurance***

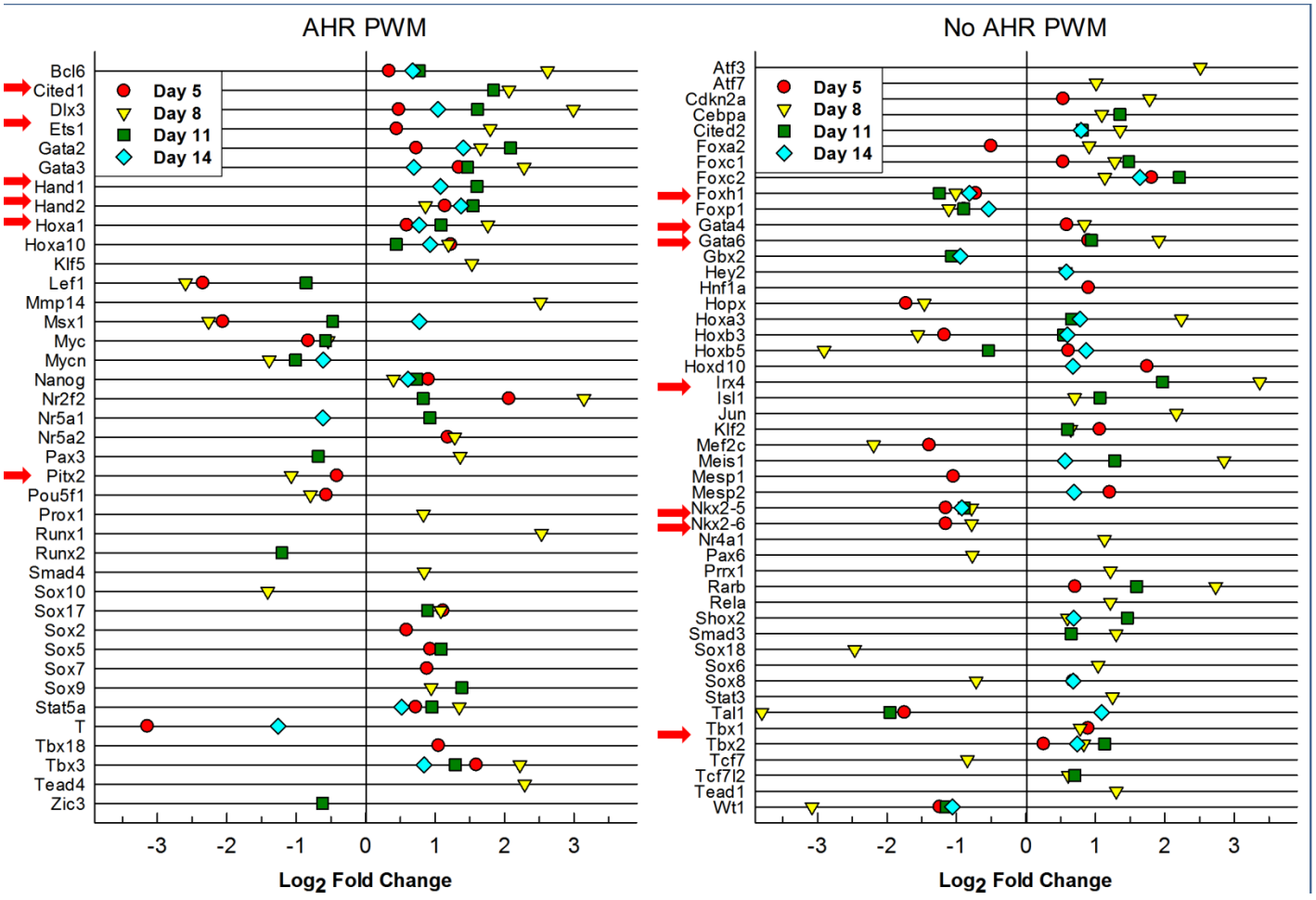
***Ahr* reinforces *Nkx2.5*-dependent haploinsufficiency**






Ligand mediated toxicity

# Many transcription factors are disrupted by TCDD in ES cells

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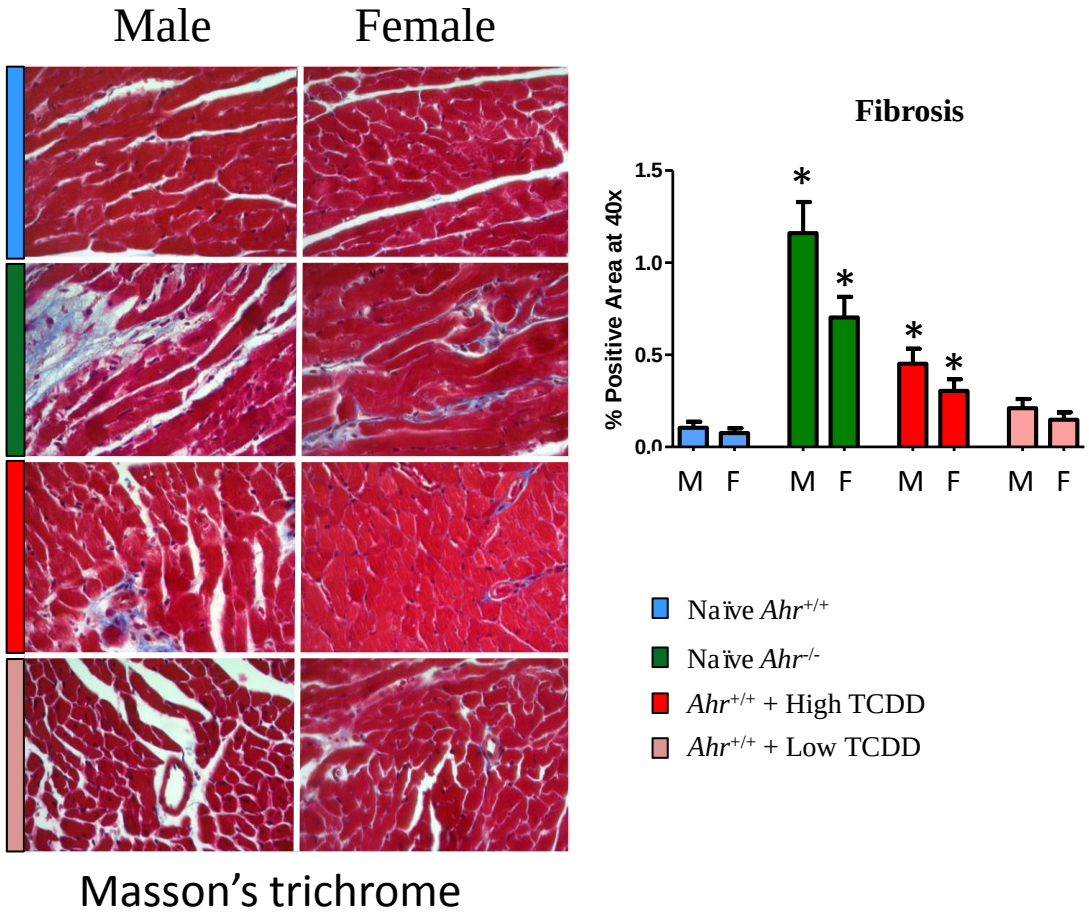
# AHR disruption during development alters the structure of the adult myocardium




Masson's trichrome