



# Histone deacetylase inhibitors as new tools to generate metabolic competent long-term cultures of primary hepatocytes

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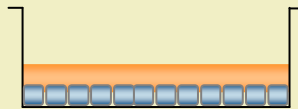
**Innovation for Sustainable Production 2008**

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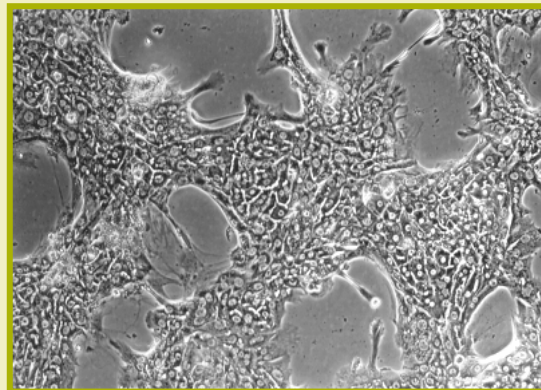


## IMPORTANT FACTORS – LONG-TERM CULTURES

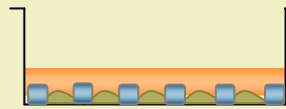
complex culture media



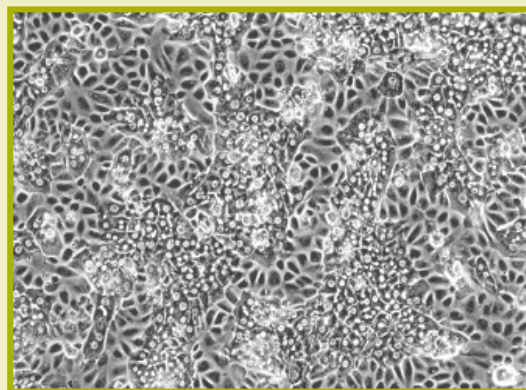
Conventional monolayer



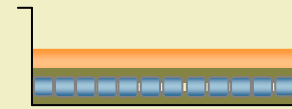
cell – cell contacts



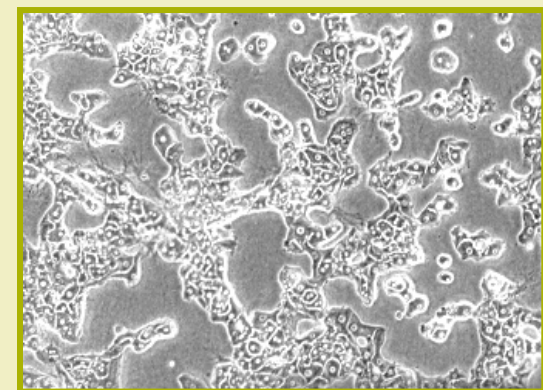
Co-culture



cell – ECM contacts

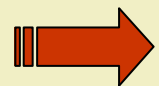
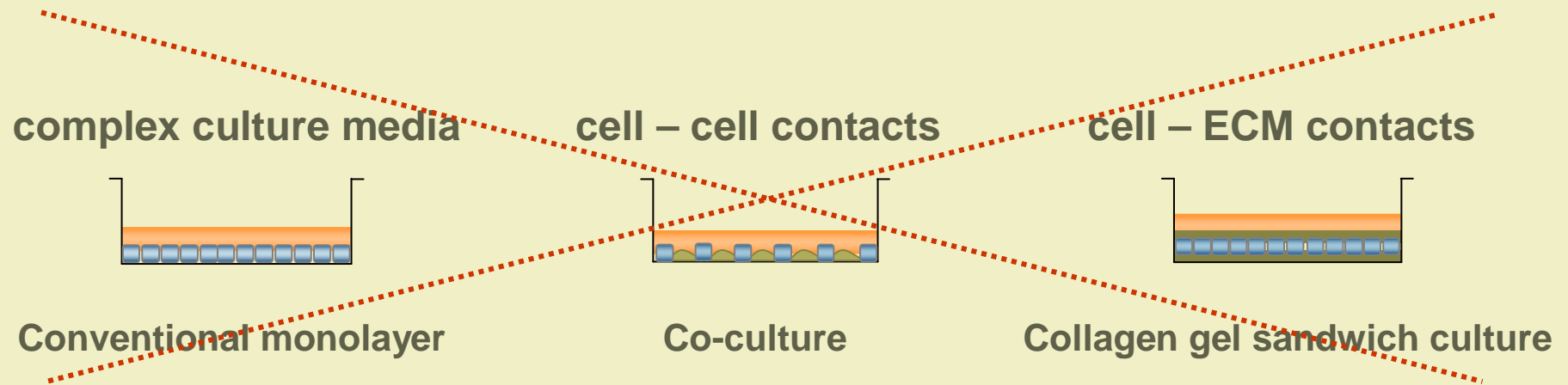


Collagen gel sandwich culture





## IMPORTANT FACTORS – LONG-TERM CULTURES

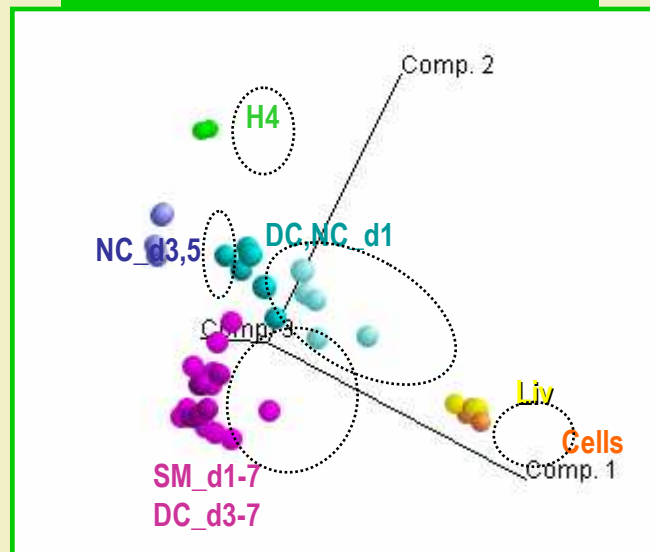


**IDEAL LONG TERM CULTURE SYSTEM DOES NOT EXIST !**

- loss of phase I and phase II biotransformation capacity
- general dedifferentiation

## TEMPORAL STABILITY OF HEPATOCYTE CULTURES

PCA - RG\_U34A - QS p0.06



Gene expression profile (present genes)  
in rat hepatocytes

- liver
- freshly isolated hepatocytes (cells)
- culture on uncoated plates (NC)
- dry collagen culture (DC)
- sandwich culture (SM)
  
- H4 hepatoma cells

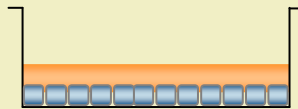


Some cultures tend to stabilize but on a very different expression level as compared to the liver *in vivo*



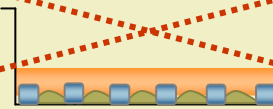
## IMPORTANT FACTORS – LONG-TERM CULTURES

complex culture media



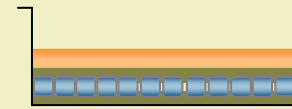
Conventional monolayer

cell – cell contacts



Co-culture

cell – ECM contacts

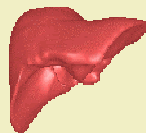


Collagen gel sandwich culture

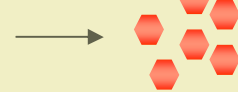
**MIMICKING *IN VIVO* MICROENVIRONMENT IS INSUFFICIENT !**

→ LIMITED LIFE-SPAN (1 – 2 weeks)

→ DEDIFFERENTIATION: loss of biotransformation capacity



G0



G1

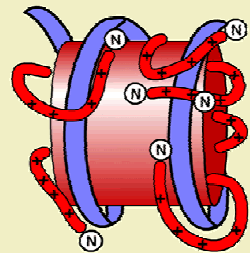
**Cell cycle re-entry :**

- loss of LETFs

- loss of CYPs

**USE OF HISTONE DEACETYLASE INHIBITORS (HDACi)**

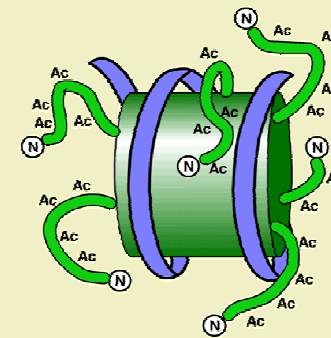
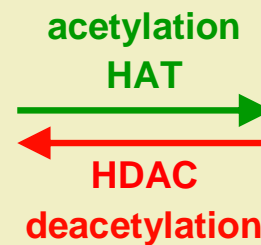
## HISTONE ACETYLATION - DEACETYLATION



Deacetylated nucleosome

condensed form  
less accessible

No transcription

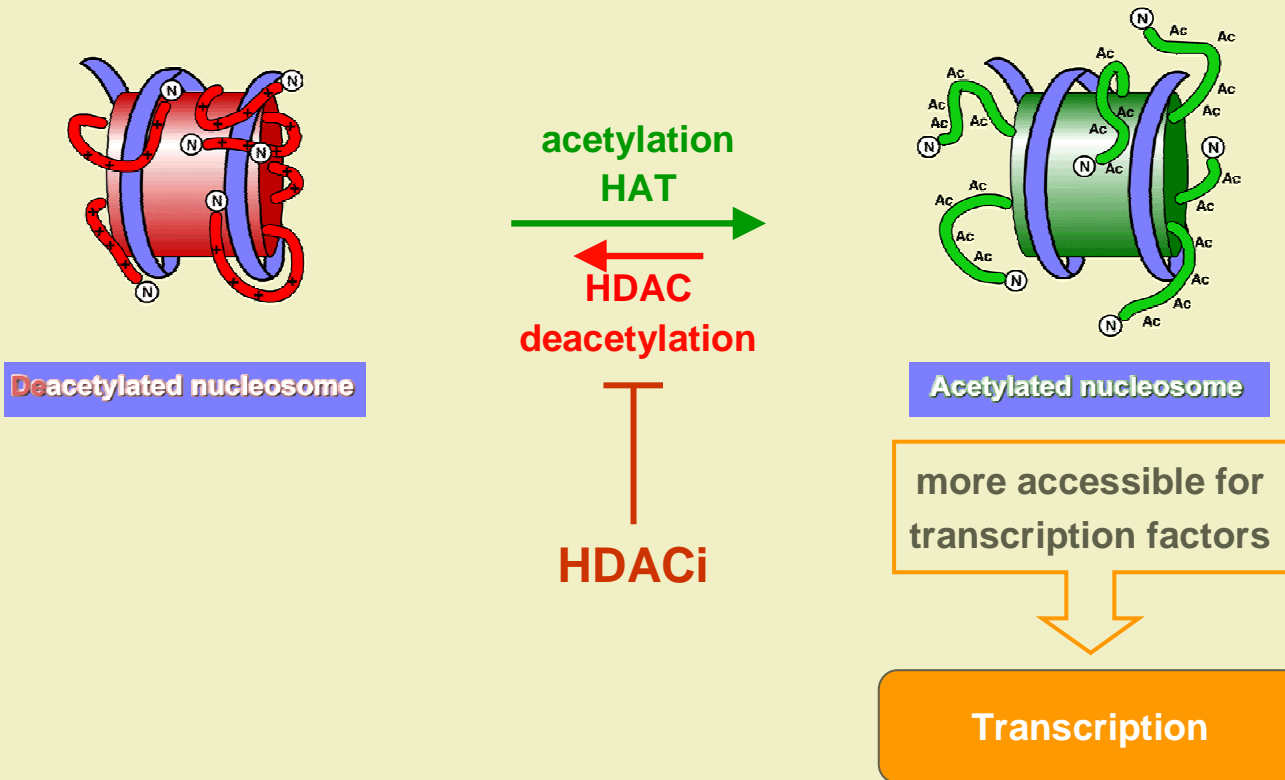


Acetylated nucleosome

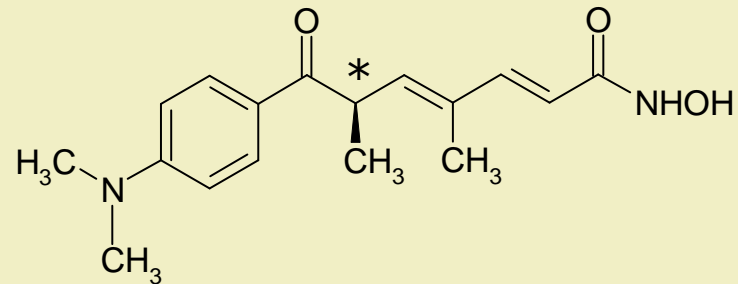
more accessible for  
transcription factors

Transcription

## HISTONE ACETYLATION - DEACETYLATION



## TRICHOSTATIN A (TSA)



- ↪ In tumor-derived cell lines: cell growth arrest, differentiation and apoptosis
- ↪ Little data available in primary cells:
  - cell cycle arrest (*Papeleu, 2003*)
  - delay apoptosis (*Vanhaecke, 2004*)
  - enhance GJIC (*Vinken, 2006*)



## KEY QUESTION

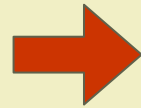
**IS THE HYDROXAMIC ACID-BASED HDACi TSA  
ABLE TO OVERCOME THE LOSS OF XENOBIOTIC  
BIOTRANSFORMATION CAPACITY IN CULTURED  
PRIMARY HEPATOCYTES ?**



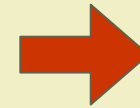
## ISOLATION AND CULTURE OF RAT HEPATOCYTES



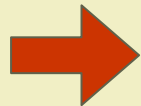
Sprague-Dawley



2-step collagenase  
perfusion



conventional  
monolayer (7d)



3 conditions: ➤ control

➤ solvent-control (ethanol)

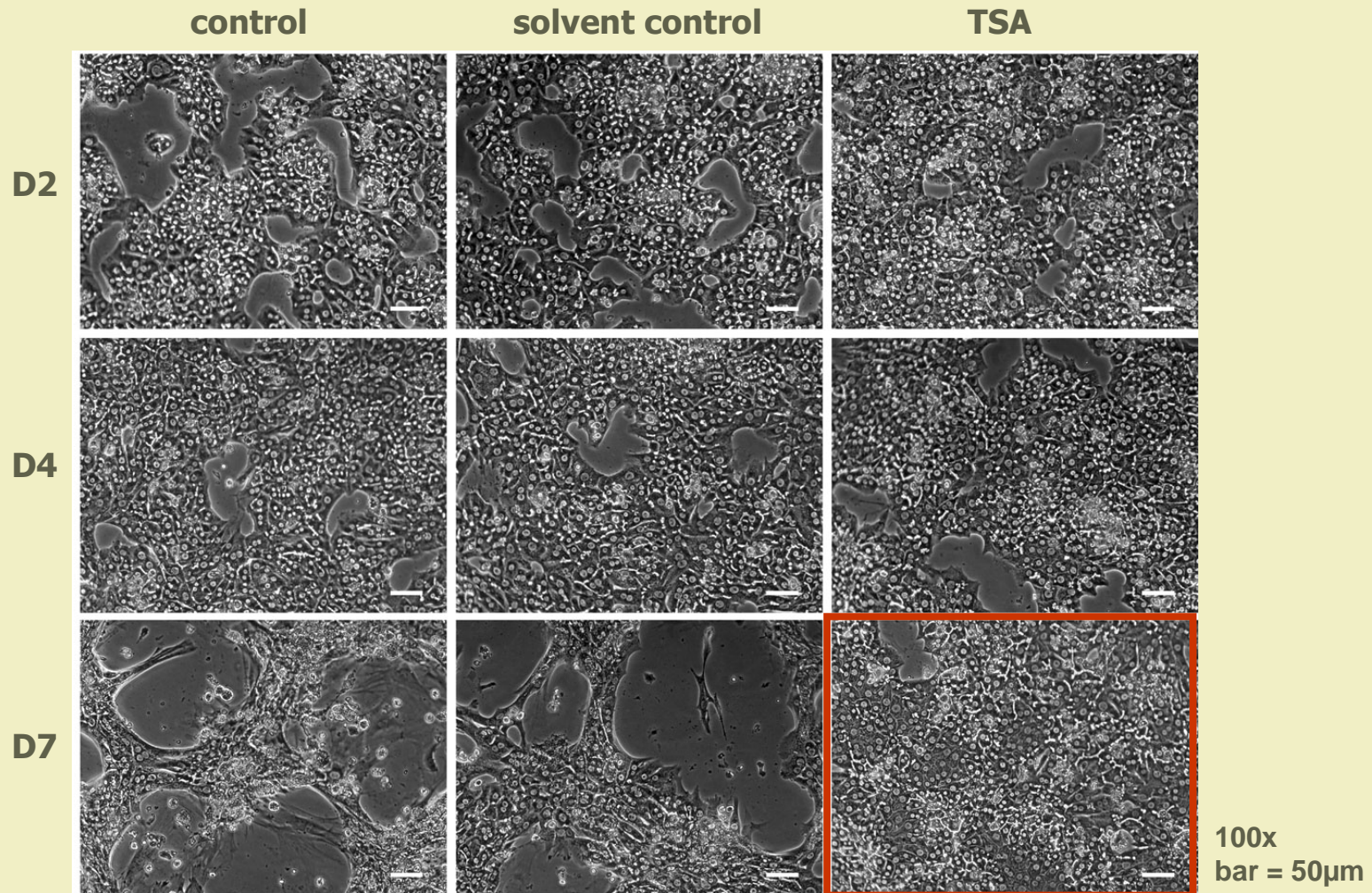
➤ TSA (25 $\mu$ M)

## HEPATIC FUNCTIONALITY TESTING

- Morphological examination
- Albumin secretion
- CYP enzymatic activities: EROD, PROD and testosterone 6 $\beta$ -hydroxylation
- CYP protein and mRNA expression levels: Western Blotting and qRT-PCR

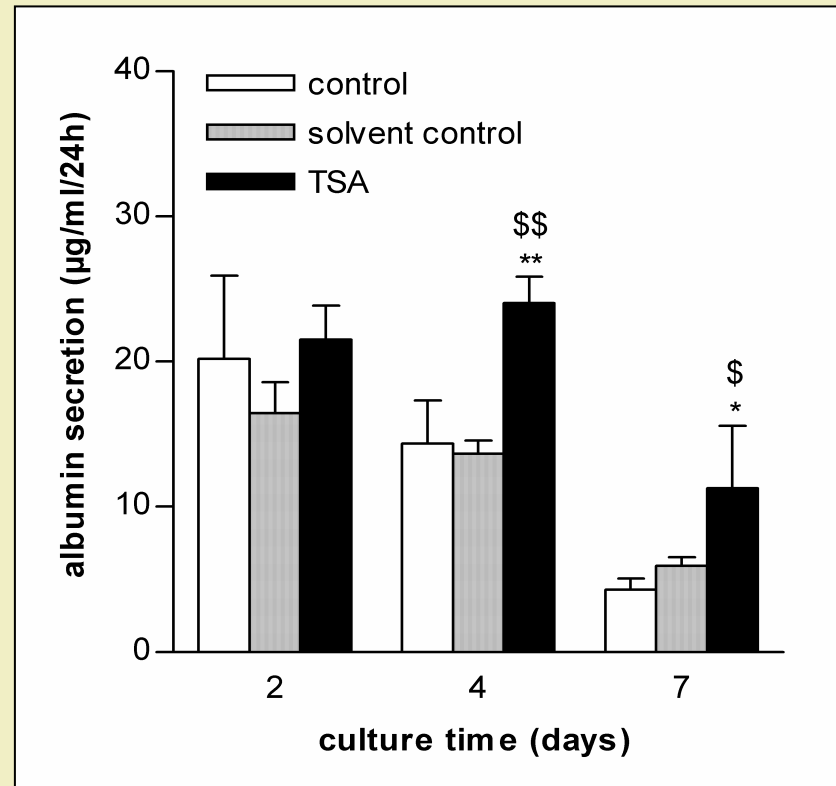


## MORPHOLOGICAL EXAMINATION





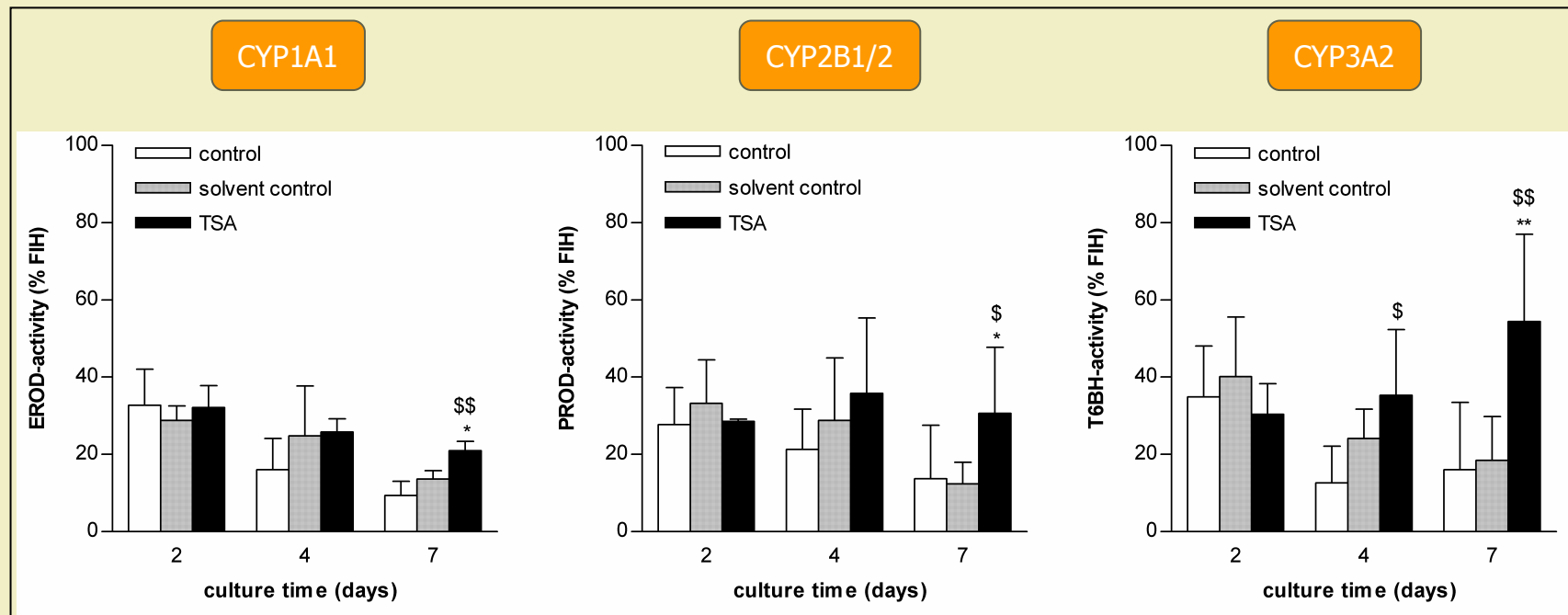
## ALBUMIN SECRETION



**HIGHER ALBUMIN SECRETION THROUGHOUT CULTURE TIME UPON EXPOSURE TO TSA**



## CYTOCHROME P450 ACTIVITIES

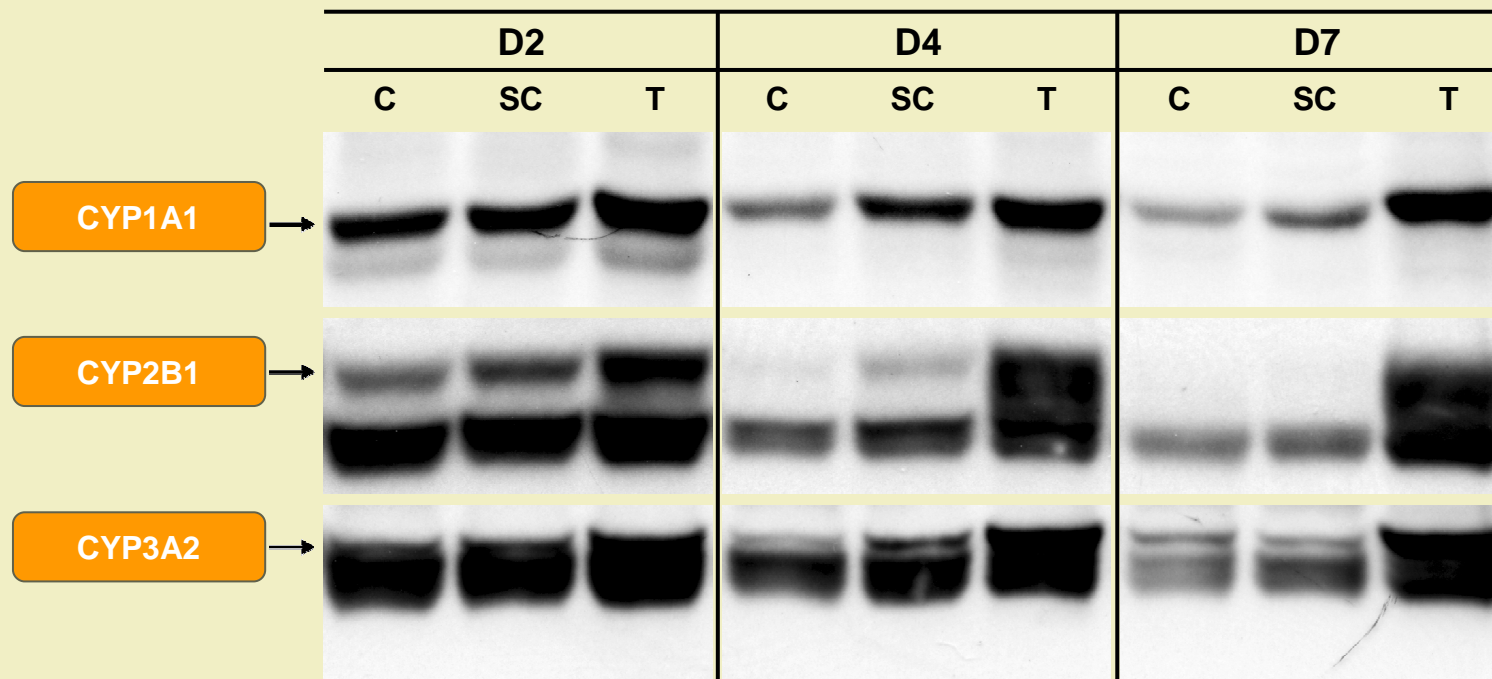


n=3; \$ p<0.05 compared to control; \* p<0.05 compared to solvent control



**BETTER PRESERVATION OF PHASE I CATALYTIC ACTIVITIES  
THROUGHOUT CULTURE TIME UPON EXPOSURE TO TSA**

## CYTOCHROME P450 EXPRESSION

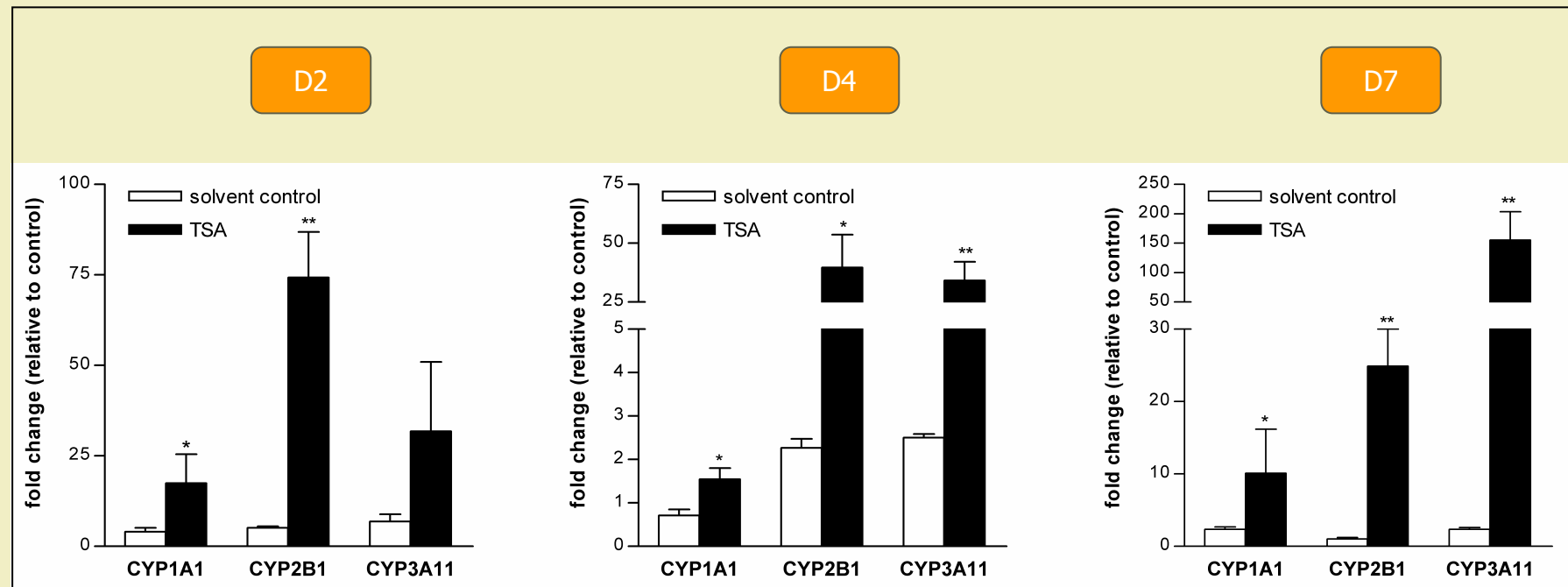


C = control; SC = solvent control; T = 25µM TSA



**CYP1A1, CYP2B1 AND CYP3A2 PROTEIN LEVELS ARE BETTER PRESERVED UPON EXPOSURE TO TSA**

## CYTOCHROME P450 mRNA LEVELS



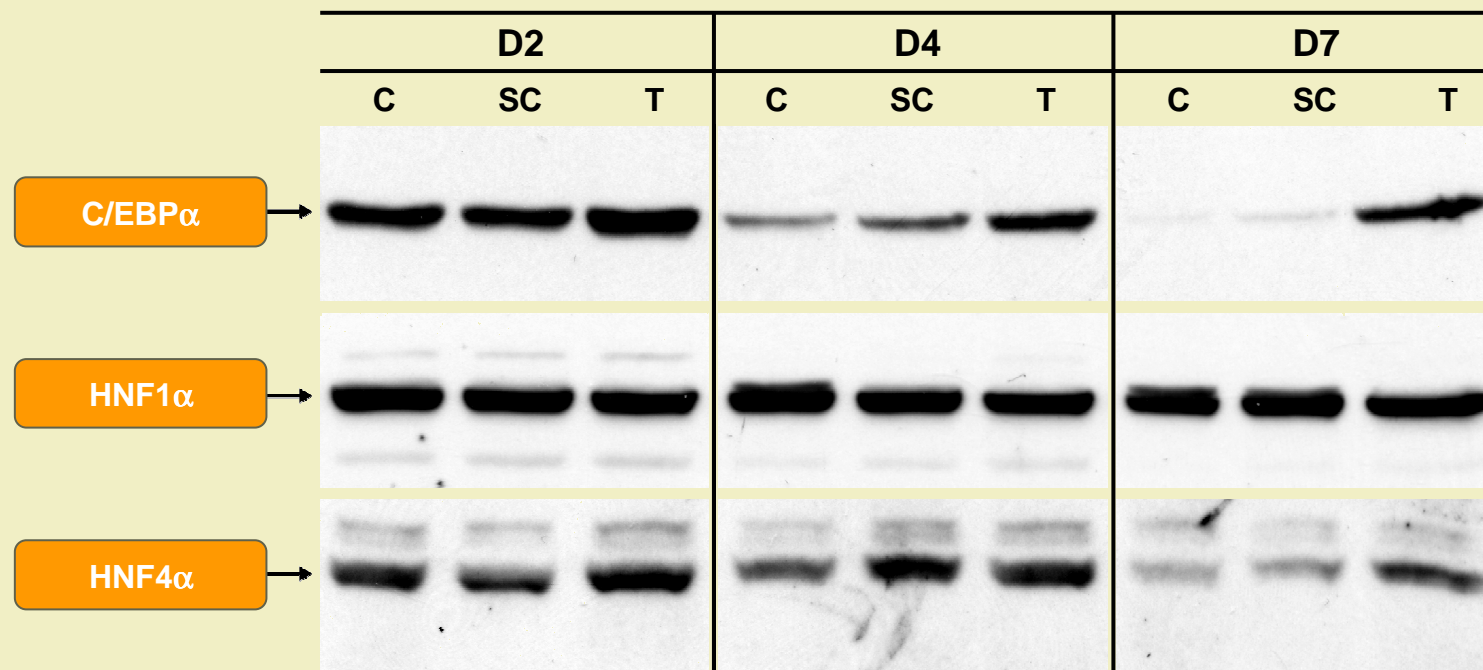
n=3; \* p<0.05 compared to solvent control



**ELEVATED mRNA LEVELS UNDERLIE THE INCREASED AMOUNTS OF CYP-PROTEINS IN TSA-EXPOSED HEPATOCYTE CULTURES**

## LIVER ENRICHED TRANSCRIPTION FACTORS (LETF's)

- ↗ indispensable for liver development, hepatocyte homeostasis and differentiation (*Cereghini, 1996*)
- ↗ Important role in loss of the hepatic phenotype in culture (*Mizuguchi, 1998*)



C = control; SC = solvent control; T = 25 $\mu$ M TSA



- ↪ **Exposure to TSA:**
  - extends viability of cultured primary hepatocytes
  - better maintains albumin secretion
  - better preserves CYP- mediated phase I biotransformation capacity
- ↪ **Effects of TSA on CYP gene expression may be mediated by controlling the expression of C/EBP $\alpha$  and HNF4 $\alpha$**

**CHROMATIN REMODELING IS AN INNOVATIVE  
APPROACH TO OVERCOME  
DEDIFFERENTIATION IN CULTURES OF  
PRIMARY HEPATOCYTES**



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